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Assessment of marine litter in the Western Adriatic Sea and its impact on marine biota

Tesi di laurea in

Habitat marini: alterazioni e conservazione

Relatore
Prof. Marco Abbiati

Presentata da
Giulia Pasquini

Correlatore
Dott. Tomaso Fortibuoni

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CONTENTS

1. INTRODUCTION

1.1 The growing problem of marine litter

There have been numerous anthropogenic-driven changes to our planet in the last half-century. One of the most evident changes is the ubiquity and abundance of litter in the marine environment (Galgani et al., 2013). Based on the definition from UNEP (2009), marine litter is defined as "any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment".

The sources of litter reaching the marine environment can be broadly categorized to whether they are land or sea based (UNEP, 2005). Land-based sources include tourism and recreational uses of the coast, domestic, agricultural and industrial activities, harbors, untreated municipal sewage and improper waste management. Sea-based sources of marine litter include merchant shipping, ferries and cruise liners, commercial and recreational fishing vessels, military fleets and research vessels, pleasure craft, offshore installations such as oil and gas platforms, drilling rigs and aquaculture sites (Galgani et al., 2013). Litter may be dumped directly into the marine environment by recreational visitors and beach-goers (Martinez-Ribes et al., 2007). Litter dropped on land, either by individuals or from commercial activities, can also enter the marine environment through a series of pathways including wind-blow, water bodies (rivers, lakes, ponds, ephemeral streams), municipal drainage systems and sewage inputs. Natural events, such as rough seas, flooding, melting of snow and heavy rainstorms, provide occasional inputs of litter into the marine environment, but may be extremely relevant. E.g. the case of the devastating tsunami that struck Japan on March 11, 2011: as a result of the disaster, the debris that the tsunami washed into the ocean reach U.S. and Canadian shores over the past several years, and it is likely that more debris will continue to arrive.

The problems generated by marine litter can be attributed to both the amount of debris generated and its nature. It is estimated that 4.8 to 12.7 million metric tons of man-made debris enter the world seas every year (Jambeck et al., 2015), persisting in marine environment for years, decades and even centuries. Materials include plastic, rubber, glass, metal, processed wood, paper, textile. According to its nature, weight and shape, marine litter can float on sea surface, be transported on shores or sink on the seafloor and accumulate.

Amongst the debris usually found in the marine environment, plastic is the most abundant type, covering sometimes up to 95% of the waste that accumulates on shorelines, the sea surface and the seafloor (Galgani et al., 2015). Plastic is a relatively new artificial material, which has been accumulating in the marine environment since 1940s (O'Brine and Thompson, 2010). Due to the fact that plastic is lightweight, non-degradable (and therefore durable), cheap, and easily producible, it has become massively popular among manufacturers and consumers. Unfortunately, the non-degradable and lightweight properties have made plastic one of the most problematic persistent pollutants in the marine environment. While the former feature ensures its lasting existence, the latter guarantees its wide dispersion in the marine environment, leading to several ecological and socio-economical impacts (Fauziah et al., 2015).

1.2 The impact of litter on the marine environment

Besides the aesthetic problem, marine litter has various potentially harmful implications on the marine environment. Threats to marine life are primarily mechanical due to entanglement in packaging bands, synthetic ropes, lines or nets, and ingestion of plastic debris (Laist, 1987a). Entanglement in marine debris has been reported for pinniped species, cetaceans, all seven species of marine turtles, and more than 56 species of marine and coastal birds (Katsanevakis et al., 2007). One of the most problematic threat is the presence of derelict or discarded fishing gear (nets, traps and pots), which may continue to "fish" for years. This process, termed "ghost" fishing, can damage benthic habitats and entangle both benthic and mobile fauna, including endangered species (Donohue et al., 2001). It is estimated that 10% of all litter entering the oceans annually consists of so-called ghost nets (Macfadyen et al., 2009).

Ingestion of anthropogenic debris occurs in several marine organisms: at least 43% of existing cetacean species, all species of marine turtles, approximately 44% of the world's seabird species, and many species of fish have been reported to ingest marine litter, either because of misidentification of debris items as natural prey or accidentally during feeding behavior (CBD, 2012). In some cases, effects of plastic fragments and other anthropogenic materials may be directly responsible for the obstruction of digestive tracts and finally death from starvation and debilitation (Laist, 1987b). To make matters worse, plastics gradually break down to microscopic sizes, becoming available to a wide range of vertebrates and invertebrates as deposit feeders, filter feeders, scavengers, and may enter the food chain (Thompson et al., 2004). Concerns refer to the evidence that plastics, besides having many added chemicals, also adsorb toxic pollutants (including polychlorinated biphenyls - PCBs and dichlorodiphenyltrichloroethane - DDT) from the surrounding water, which bioaccumulate in marine organisms once ingested, with potential consequences even on human health (Meeker et al., 2009).

Other known impacts of marine litter include alteration, damage and degradation of benthic habitats, i.e. the smothering of fauna (Katsanevakis et al., 2007), the inhibition of gas exchange between the overlying waters and the pore waters of the sediments (Goldberg, 1997), as well as the transport and the spread of alien species, including algae associated with red tides (Barnes and Milner, 2005).

From a socio-economic point of view, harms by marine litter can include the reduction of ecosystem goods and services (Galgani et al., 2013). Litter stranded on beaches is an offence to the visual and aesthetic sensitivities of tourists and local visitors, resulting in a loss of recreational value of coastal areas, with important consequences on the local economy (Munari et al., 2015). Furthermore, sanitary and medical waste may cause injuries and be a risk to human health (Ivar do Sul and Costa, 2007). Floating debris may get entangle in boat propellers or clog cooling water intakes, causing damages to boat motors and troubles to navigation (Aliani et al., 2003) and break fishing gears, with impacts on subsistence of fishermen (Nash, 1992).

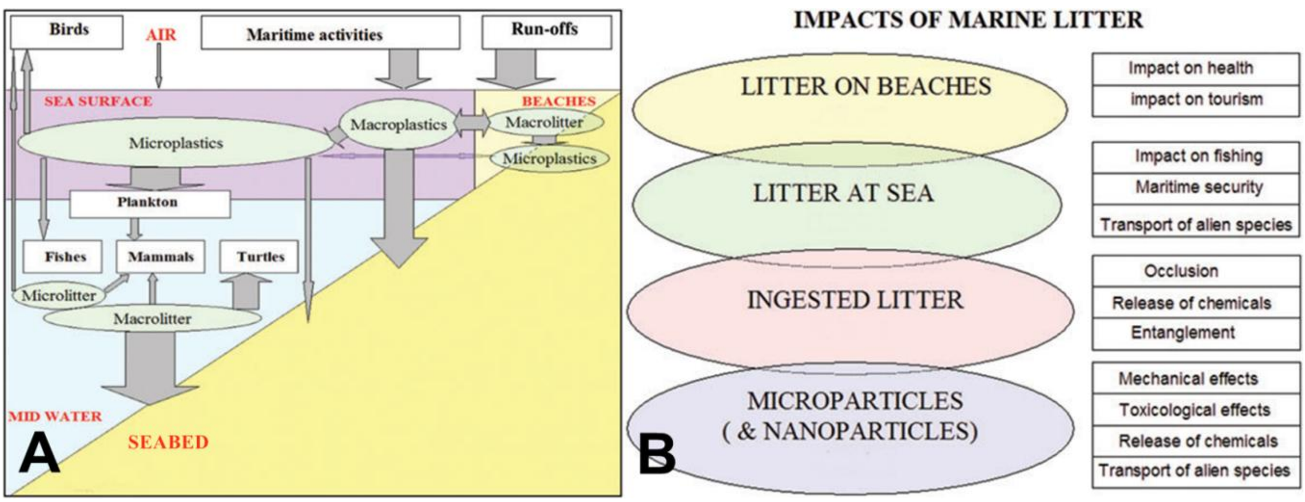


Figure 1.2.1 A: Schematic cycle of litter at sea and pathways of litter entering the marine environment. Grey arrows represent relationships between different habitats and biological entities, and thickness symbolizes the extent of interactions. **B:** Major impacts of marine litter on main environmental and biological compartment.

1.3 Marine litter within the European Marine Strategy Framework Directive and DeFishGear Project

Marine litter is a descriptor listed by the EU Marine Strategy Framework Directive (MSFD). The main goal of the MSFD is to achieve Good Environmental Status (GES) in the EU marine waters by 2020. In order to help Member States in interpreting what GES means in practice, the Directive sets out, in Annex I, eleven qualitative descriptors which describe what the environment will look like when GES has been achieved. Focusing on marine litter, Descriptor 10 describes the following criteria for Good Environmental Status: "*Properties and quantities of marine litter do not cause harm to the coastal and marine environment*" (European Commission, 2008). A number of indicators have been suggested to support the monitoring of marine litter, these include:

- trends in the amount of litter washed ashore or on coastlines;
- trends in the amount of litter in the water column and on the sea-floor;
- trends in the amount, distribution and composition of micro-particles;
- trends in the amount and composition of litter ingested by marine animals.

The European Commission has commissioned several projects in order to obtain information on abundance, composition, sources and fate of marine debris and eventually provide management measures to reach the GES for marine litter at a regional scale. In recent years, research efforts have significantly improved the knowledge on the issue of marine litter; however, the whole field has not adopted standardized monitoring procedures yet (Lippiat et al., 2013).

DeFishGear project (Derelict Fishing Gear Management System in the Adriatic Region; www.defishgear.net) arose in 2013 from the need of a coordinated and harmonized marine litter monitoring plan in the Adriatic Sea, and ultimately provide a strategic input to regional efforts in successfully achieving the GES in the Mediterranean Sea. Co-founded by the IPA Adriatic Cross-border Cooperation Programme, the project took up the challenge to address the lack of reliable scientific data on marine litter in the Adriatic Sea, involving research institutes, national and local authorities and NGOs from all seven countries facing the basin (Figure 1.2.2), developing harmonized monitoring protocols, carrying out surveys in all environmental compartments (beach, sea surface, seafloor, biota) and promoting joint actions towards a litter-free Adriatic.

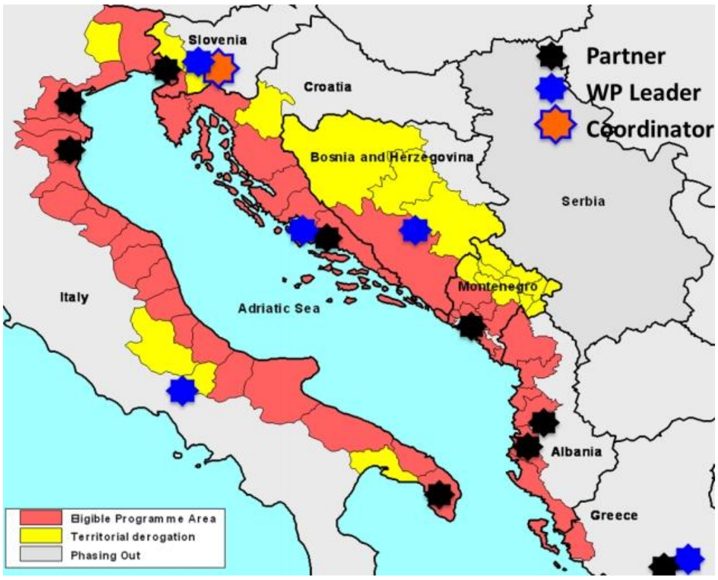


Figure 1.2.2 DeFishGear project partnership. **Coordinator:** National Institute of Chemistry, Ljubljana, (Slovenia). **Project partners:** Italian National Institute for Environmental Protection and Research, Chioggia (Italy); Ca' Foscari University of Venice (Italy), Mediterranean Consortium, Rome (Italy); Regional Agency for Environmental Protection in the Emilia-Romagna Region, Cesenatico (Italy); Euro-Mediterranean Centre on Climate Change, Lecce (Italy); Institute for Water of the Republic of Slovenia, Ljubljana (Slovenia); University of Nova Garcia, the Laboratory for Environmental Research, Nova Garcia (Slovenia); Institute for Oceanography and Fisheries, Split (Croatia); Public Institution RERA SD for Coordination and Development of Split Dalmatia County, Split (Croatia); Hydro-Engineering Institute of the Faculty of Civil Engineering, Sarajevo (Bosnia and Herzegovina); Institute of Marine Biology, University of Montenegro, Kotor (Montenegro); Agricultural University of Tirana, Laboratory of Fisheries and Aquaculture, Tirana (Albania); Regional Council of Lezha, Lezha (Albania); Mediterranean Information Office for Environment, Culture and Sustainable Development, Athens (Greece); Hellenic Centre for Marine Research, Anavyssos (Greece).

1.4 Data on beach litter in the Adriatic region

Marine litter stranded on beaches is found along all coasts worldwide and has become a permanent cause for concern. Beaches and shores act as depositional environments, and are very sensitive to accumulation of marine litter, that can reach up to an average of 15.3 (1.2–78.3) items/m^2 on some beaches of South East Asia (Smith, 2012). Beach litter data derive from scientific surveys and/or clean-up campaign and are collected following different approaches and protocols. These surveys allow a large sampling effort at a low cost, since they require minimal equipment and inexperienced staff, and can be carried out in most weather conditions (Rees and Pond, 1995).

Beach litter has been little studied in the Adriatic area, and particularly along the Italian coasts. So far, only one study dealing with the assessment of marine litter abundance along Adriatic Italian shores was published (Munari et al., 2015). Data on the amounts, distribution and composition of marine litter along the coastline of the Adriatic Sea generally come from clean-up campaigns carried out by environmental NGOs (such as Legambiente, WWF, etc.) in collaboration with local authorities and municipalities. In most cases, data are limited to total quantity of marine litter collected, or amounts of litter collected by material type, without any further classification of types of items (Vlachogianni and Kalampokis, 2013). Furthermore, different Organisations and local associations use different tools and methods, making it impossibile to analyse and compare data. However, surveys revealed that the greater majority of stranded debris was made of plastic (80%), a category of litter dominant in beaches all over the world (Legambiente, 2015; Munari et al., 2015). Most common plastic items are sheets, polystyrene pieces and hard fragments, whilst whole stranded debris include bottles, caps, cigarette butts, fishing related items and picnic items, indicating that most marine litter comes from local sources and direct use on the beach (Munari et. al, 2015).

1.5 Data on benthic litter in the Adriatic region

The presence, abundance and composition of anthropogenic debris on the seafloor is much less investigated compare to sea surface and shores, because of sampling difficulties, inaccessibility and costs (Galgani et al., 2013). Among benthic litter monitoring methodologies, trawling is considered the most effective. However, since nets were primarily designed for fishing purposes, sample bias and underestimation of benthic litter quantities may occur (Spengler and Costa, 2008).

In the Adriatic Sea, information on the deposits of macro litter on the seafloor is fragmented. Surveys have been done by voluntary divers (e.g. Ljubec, 2013), submersibles, remote operated vehicles (Galgani et al., 2000), and trawl surveys in shallow (Galil et al., 1995; Galgani et al., 2000) and deep waters (Petović and Marković, 2013). Data regarding litter on the seafloor in the Adriatic Sea are also available from the SoleMon project (Solea Monitoring - Rapido trawl survey in the Northern Adriatic Sea) (Strafella et al., 2015), carried out since 2005 in the Northern and Central Adriatic Sea. Plastics is the most abundant component of the macro-debris on the sea floor, to an extent similar to that observed in the floating litter and beach debris. It accounts for about the 70% of the total litter collected, corresponding to a mean abundance of 378 items/Km^2 (Galgani et al., 2000) and 34 ± 4 kg/km^2 (Strafella et al., 2015).

1.6 Impacts of litter on marine biota in the Adriatic region

The ingestion of anthropogenic debris by marine wildlife, ranging from zooplankton to megafauna (fish, seabirds, sea turtles, and marine mammals) has been widely documented. As for fishes, the first report of marine debris ingestion was published by Carpenter et al. (1972) and described the presence of plastic particles in larvae and adult fish. In recent decades ingestion of plastic debris has been documented in many species of fish, rays and sharks (e.g. Jantz et al., 2013; Lusher et al., 2013; Miranda and de Carvalho-Souza, 2015; Romeo et al., 2015), and the number of records is still growing.

So far, few studies were carried out in the Mediterranean region, highlighting the impacts of debris on fish populations. Anastasopoulou et al. (2013) documented the occurrence of marine debris in five deep-water fish species inhabiting the Eastern Ionian Sea out of the twentysix species collected. Ingested items consisted primarily of plastics (86.5%), including hard pieces, bags and fishing gears fragments (Anastasopoulou et al., 2013). Recently, Romeo et al. (2015) focused, for the first time, on the occurrence of plastic debris in the stomach content of three Mediterranean top predators: *Xiphias gladius* (swordfish), *Thunnus thynnus* (blue fin tuna), *Thunnus alalunga* (albacore). Results highlighted the ingestion of plastic in the

18.2% of samples, providing an important contribution to the knowledge of its occurrence in these commercial fish, given also their relevance in human diet. Although this phenomenon has larger proportion in some oceanic waters characterized by convergence currents, plastic debris was also found in the guts of organisms living in the Adriatic Sea, mainly cetaceans and turtles (Mazzariol et al., 2011; Lazar and Gračan, 2011). Very limited data are available on commercial fish species, (Avio et al., 2015), despite the Adriatic Sea represents one the major fishing ground in the whole Mediterranean basin (Mannini et al., 2004).

2. AIMS OF THE STUDY

This thesis was carried out in the framework of DeFishGear project with the aim to:

I) Assess the quantity and the quality of marine litter occurring on beaches along the Western coastline of the Adriatic Sea.

II) Quantify litter abundance, composition and spatial distribution on the seafloor of the Northern and Central Adriatic Sea; estimate the contribution of human activities to seafloor litter distribution and investigate the relationships among the main sources.

III) Verify through stomach and gut contents analysis if fish with different habits and habitats, inhabiting the Northern Adriatic coastal waters, ingest debris and to what extent.

The ultimate goal is to provide insights into possible approaches to manage marine litter deposition in different environmental compartments, and enhance the knowledge regarding the incidence and impacts of marine litter on fish species which are commonly caught and intended to human consumption.

3. MATERIALS AND METHODS

3.1 Study area: the Adriatic Sea

The Adriatic Sea is a semi-enclosed basin within the Mediterranean Sea. It extends over 138,000 km^2 (Buljan and Zore-Armanda, 1976) and it is characterized by Northern, Central and Southern sub-basins with decreasing depth from the south toward the north (Mannini et al., 2004). The northern section is very shallow and gently sloping, with an average depth of about 35 m, while the central and the southern are on average 140 m deep, with the two Pomo Depressions reaching 260 m (Strafella et al., 2015). The majority of the seabed is located on the continental shelf and is covered by sandy and muddy sediment with different grain size and composition.

Sediments deposited in the Adriatic Sea generally come from the Italian northwest coast, being carried by the large number of rivers discharging into it, i.e. the Po (the most relevant), Adige, Piave, Reno and Brenta. The volume of sediments carried by rivers on the eastern shore, for instance Neretva and Cetina, is negligible, because these sediments are mostly deposited at the river mouths.

Two main currents dominate the Adriatic circulation: the West Adriatic Current (WAC) flowing toward South-East along the western coast, and the East Adriatic Current (EAC) flowing North-East along the eastern coast. Two main cyclonic gyres occur, one in the northern part and the other in the South (Strafella et al., 2015). Bora (from North-East) and Sirocco (from South-East) are the major winds blowing over the Adriatic Sea.

The Adriatic basin is heavily stressed by many human activities. Heavy marine traffic derives from commercial, fishing and recreational activities (Carić and Mackelworth, 2014). It hosts hundreds offshore platforms by the oil and gas industries. Fishery and mussel aquaculture along the Italian coast, and fish farming along the Croatian coast, are crucial economical sources for countries facing the basin, and strongly affect the features of the basin (Strafella et al., 2015).

Six countries, whose coastline development differs greatly, border the Adriatic (Mannini et al., 2004): Italy, Slovenia, Croatia, Bosnia-Herzegovina, Montenegro, Albania.

The western Adriatic coast, possessing the longest beaches in Europe, is home of a thriving tourism industry (Munari et al., 2015). Most of beaches, which are State property, are given in concession to entrepreneurs, and structured in ‘‘lidos” with restaurants and leisure options. Some stretches of beach, distinguished by their naturalistic value, are kept free from lidos (free access beaches) and relatively free from beach cleanups. The Italian Adriatic coast is characterized by intense urbanization, being 8 out of the total 20 regions and 121 municipalities (over 3,476,800 residing inhabitants) directly facing the basin (Romano and Zullo, 2014).
All these features (river discharge, shipping lanes, fishery, aquaculture and coastal urbanization) are critical in determining the abundance, composition and distribution of marine litter on the seafloor and coastline of the Adriatic Sea.

3.2 Beach litter monitoring

3.2.1 Site selection

Monitoring sites were selected taking into consideration the folowing criteria (Hanke et al., 2013; Vlachogianni et al., 2015):

- a minimum length of 100 m;
- sandy composition and low to moderate slope (1.5 – 4.5º);
- do not host lidos;
- open access to the sea (not blocked by breakwaters or jetties) such that litter coming from water is not screened by anthropogenic structures;
- accessible to survey teams year round.

Along the Western Adriatic coast, three free access beaches, part of the system of the Natura 2000 Italian network, were chosen as monitoring sites: Rosolina Mare (RO) and Boccasette (RO), included into the Veneto Regional Parks of the Po River Delta, Torre del Cerrano (TE), within the homonymous Marine Protected Area (Fig. 3.2.1 A).

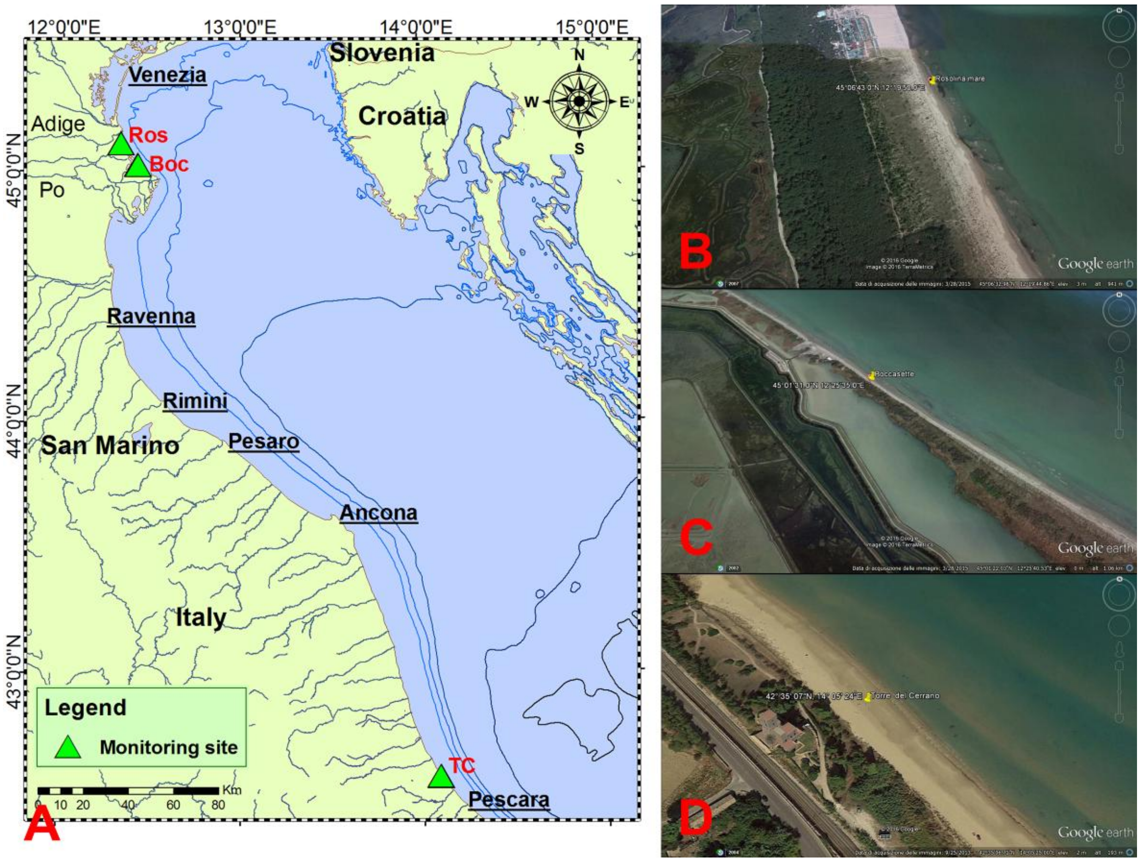


Figure 3.2.1. (A) Location of the beaches (Ros: Rosolina mare; Boc: Boccasette; TC: Torre del Cerrano). Site morphology of Rosolina mare (B), Boccasette (C) and Torre del Cerrano (D).

Rosolina mare (45° 06’ 43”N; 12° 19’ 50”E) is a 100% fine sandy beach surrounded by 172 ha of pinewood (Fig. 3.2.1 B). The most relevant trait is its proximity to the mouth of Adige river (length: 409 km; mean water discharge: 202 m^3/s), the second longest and the third largest Italian river (Chiogna et al., 2016), and the proximity to extensive aquaculture (mussel farming) installations. The site is a common summer destination, especially for local inhabitants of the nearby, being the nearest town (Rosolina, 6,510 inhabitants) 12 km far.

Boccasette (45° 01’ 31”N; 12° 25’ 35”E) is a remote “scanno”, a narrow natural strip of sand located in the most extreme part of the Po Delta Regional Park (Fig. 3.2.1 C). The peculiarity is the vicinity to the mouth of the Po river (length: 652 km; mean water discharge: 1,540 m^3/s), the largest Italian river. The area is also subjected to intense aquaculture, being several mussel farms just 4.5 km far from the beach.

The site of Torre del Cerrano (42° 35' 07"N; 14° 05' 24"E) is located within the Zone B of the homonymous Marine Protected area “Torre del Cerrano”, along the Central Italian Adriatic coast (Fig. 3.2.1 D). The site is a popular tourist destination thanks to the ancient submerged port of Atri, the medieval tower, the shady pinewood and the pristine dune environment.

Beach characteristics are summarized in Table 3.2.1.

Table 3.2.1. Characteristics of study sites.

Site [Natura 2000 network code]	**Beach length (m)**	**Beach width (m)**	**Distance to the closest river mouth (Km)** [Name, length, flow]	**Distance to the closest town (Km)** [Name, no. of inhabitants]	**Distance to the closest mussel farming (Km)**
Rosolina mare [IT3270004]	7,100	25-50	4.9 [Adige, 409 km, 202 m^3/s]	7.9 [Rosolina, 6,468 in.]	7
Boccasette [IT3270017]	4,400	20-25	1.8 [Po, 652 km, 1,540 m^3/s]	11.5 [Porto Tolle, 9,920 in.]	4.5
Torre del Cerrano [IT7120215]	7000	20-40	8 [Vomano, 76 km, 15 m^3/s]	4.4 [Pineto, 14,807 in.)	12

3.2.2 Monitoring methodology

Two monitoring surveys were carried out between April and July 2015, one before the beginning of the tourist season (addressed in the present study as "Spring"), and one during it (addressed as "Summer"). Two sampling units (replicates) in each beach were randomly identified, starting from the strandline to the back of the beach, until the beginning of the vegetation or dune, ranging from 600 to 1100 m^2 each (Hanke et al., 2013; Vlachogianni et al., 2015). The same sampling units were monitored in both surveys. During the sampling, all items larger than 2.5 cm in the longest dimension were collected, counted and classified according to the DeFishGear "Beach litter monitoring sheet", which consists in a master list of 159 categories grouped in eight main groups: artificial polymer materials (plastic), rubber, cloth/textile, paper/cardboard, processed/worked wood, metal, glass/ceramics, unidentified and/or chemicals. After the collection each main group was weighted.

3.2.3 Data treatment and source identification

In order to identify the major sources of waste on beaches, marine litter was classified into five categories related to the activity of origin (Ocean Conservancy, 2010), including:

- Shoreline and recreational activities: indiscriminate and intentional littering by beachgoers, tourists, picnickers, and litter carried from streets, drains and culverts (e.g., bottles, drink cans, picnic cutlery and dishes, toys, etc).

- Ocean/Waterways activities: improper handling of solid wastes from recreational and commercial fishing and shipping, aquaculture installations, military ships, cruise ships, and offshore oil and gas rigs (e.g., mussel nets, buoys, fishing lines, etc.).
- Smoking-related activities: improper disposal and littering of smoking-related materials and packaging (i.e., cigarette butts, lighters, cigarette boxes and plastic packaging).
- Dumping activities: improper disposal of building and construction materials, drums, tires, cars/car parts, household trash (such as cleaner bottles and containers, rubber gloves, etc.), and appliances.
- Medical/personal hygiene: materials discharged into sewer systems, dumped by storm drains and toilets, or left behind by beachgoers (e.g., cotton bud-sticks, syringes, tampons, etc).

The group “Uncertain source” was added to these categories, including all items whose source is indefinite (i.e., plastic fragments, plastic sheets, polystyrene pieces, etc.) or that may come from multiple sources (i.e., bags, ropes, plastic rings from bottles, etc.).

Statistical analyses were based on density data expressed in number of items/m^2 instead of linear distance (items/m) so that results do not depend on beach width/length and spatial comparisons are allowed.

3.2.4 Data analysis

Beach cleanliness was assessed through the Clean Coast Index (CCI), developed by Alkalay et al. (2007):

$$CCI = (\text{Total litter on transect}/\text{Total area of transect}) \times K,$$

where K (constant) = 20, inserted in the equation for statistical reasons.

The Index classifies beaches from ‘‘Clean” to ‘‘Extremely dirty” according to the scale shown in Table 3.2.2.

Total litter on beach and total area of beach were calculated as the mean between values of the two sampling units.

Table 3.2.2. Clean Coastal Index: value and definition for each quality class (from Alkalay et al., 2007).

Quality	Value	Definition
Very clean	0–2	No litter is seen
Clean	2–5	No litter is seen over a large area
Moderate	5–10	A few pieces of litter can be detected
Dirty	10–20	A lot of litter on the shore
Very dirty	20+	Most of the beach is covered with litter

Spatial differences for each season in litter composition and density were investigated through the non-parametric multivariate permutational analysis of variance (PERMANOVA; Anderson et al., 2008), using a one-way experimental design with site as a random factor (levels: 3). It was not possible to analyse date by seasons since samples were not independent. The resemblance matrix was based on Bray-Curtis similarity index, since it ignores double zeros, which might result from different environmental reasons, or lower sampling effort, rather than from an actual similarity. For each season, analyses were performed on untransformed density data and on data grouped by source. PERMANOVA calculates a pseudo-F statistic that is directly analogous to the traditional F-statistic for multifactorial univariate ANOVA models. An empirical P-value ($\alpha = 0.05$) was provided using asymptotical Monte Carlo P-values, since there were not enough possible permutations to get a reliable test using unrestricted permutation. The analyses were performed using PRIMER 6.1 with PERMANOVA+ (Anderson et al., 2008).

For each season, differences among sites in the densities of items aggregated by source were also investigated using the Kruskal–Wallis test, the non-parametric equivalent of the one-way analysis of variance (ANOVA) (Kruskal and Wallis, 1952), using STATISTICA 7. Each source category was individually tested.

Non-parametric tests to analyze results were use in order to avoid problems with the asymmetrical distribution of the data and to maximize the robustness of the results.

3.3 Benthic marine litter monitoring

3.3.1 Sampling methodology

Monitoring was carried out in the FAO Geographical Sub-Area 17 (GSA 17: northern and central Adriatic). The investigated area has a surface of 36,742 km^2 and extends from the Italian coast to the 12 nm limit of the Croatian national waters, and from 8 to 100 m depth (Fig. 3.3.1).

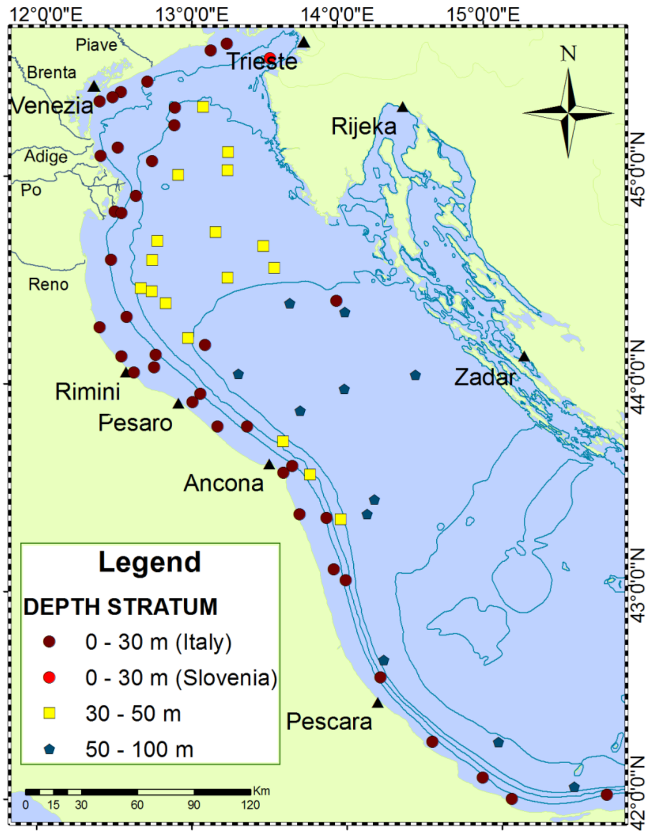


Figure 3.3.3. Stations sampled in the SoleMon survey in 2014. Stations are classified according to the depth stratum: 0-30 m (brown and red circles), 30-50 m (yellow squares), 50-100 (blue pentagons).

Monitoring survey was conducted within the framework of the SoleMon project during fall 2014, by the Institute of Marine Sciences of the National Research Council (CNR-ISMAR, Italy) in cooperation with the Italian National Institute for Environmental Protection and Research (ISPRA, Italy), the Institute of Oceanography and Fisheries (IOF, Croatia), and the Fisheries Research Institute of Slovenia (FRIS, Slovenia). The main objective of the SoleMon project is to provide information for stock assessment and fishery management on benthic and demersal commercial species, primarily common sole (*Solea solea*). However, anthropogenic waste data are also collected (Strafella et al., 2015).

The survey was performed with the research vessel “G. Dallaporta” (length: 35.3 m; tonnage: 285 TJB; power: 809 kW), equipped with the *rapido* trawl, a modified beam trawl commonly used by the Italian fishermen in the Adriatic to catch flat fish and other benthic species (Fig. 3.3.2). The gear consists of a rigid mouth rigged on the lower leading edge with 46 iron teeth and 4 skids. An inclined wooden board fitted to the front of the iron frame keeps the gear always in contact with the seabed. The fixed size of the mouth of the gear allows to exactly know the surveyed area at each haul. The net is made of polyamide and is protected in its lower side by a reinforced rubber diamond-mesh net. The codend was 2.7 m long and had 40 mm stretched-mesh size (SoleMon, 2012).

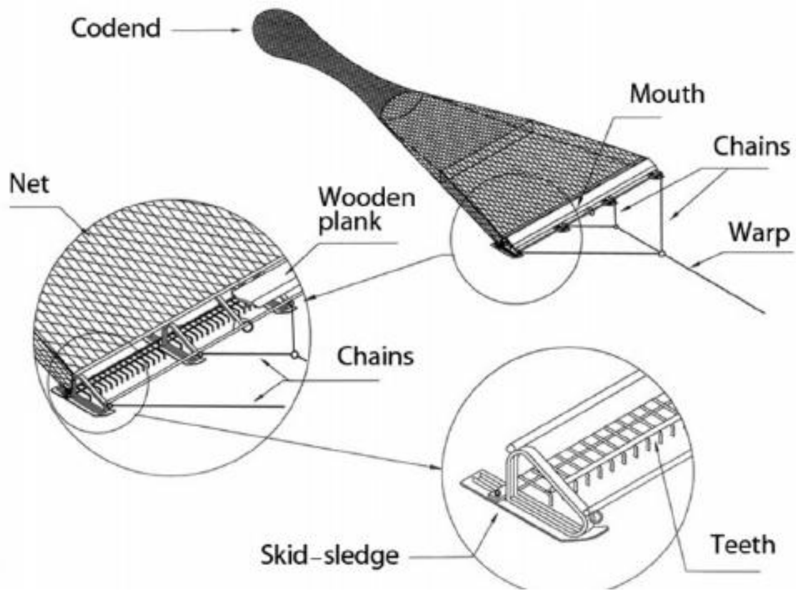


Figure 3.3.4. Scheme of the *rapido* trawl used during the SoleMon survey.

A total of 67 stations were sampled (Fig. 3.3.1). Hauls were distributed over the area following a depth-stratified random design (5-30 m: 39 stations; 30-50 m: 17 stations; 50-100 m: 11 stations). The number of stations in each stratum is proportional to its surface (Table 3.3.1). Hauls were carried out during daylight (from 30 minutes after sunrise to 30 minutes before sunset). At each station, the vessel towed two gears (RAPIDO A and RAPIDO D) simultaneously at an average speed of 5.5 knots and at a constant depth (Fig. 3.3.3). The hauls usually lasted 30 minutes, starting when the gear settles on the bottom and ending when hauling commences. Depending on circumstances, the towing time was reduced in order to avoid the overloading of the nets. In such cases, the haul was repeated and the catches were pooled together (Strafella et al., 2015).

Table 3.3.1. Strata characteristics.

Country	Stratum	Depth (m)	Surface (km²)	Area	No. of stations
Italy-Slovenia	1	0-30	11,361	Northern-Central Adriatic Sea	39
Italy - Croatia	2	30-50	8,410	Northern-Central Adriatic Sea	17
Italy - Croatia	3	50-100	22,466	Northern-Central Adriatic Sea	11

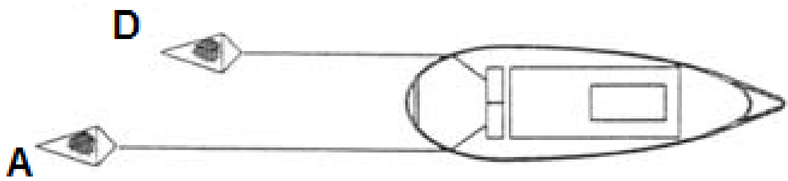


Figure 3.3.5. Scheme of gear position during the haul.

After trawling, litter items from RAPIDO D were separated from the catch, weighted, photographed and classified by a list of 46 categories, grouped in 6 major groups according to the nature of the material: plastic, metal, rubber, glass, natural (wood and paper), and mix (textile and other materials).

3.3.2 Data classification

In order to detect major contributions of waste on the seafloor, marine litter was additionally classified into three categories based on its source (land, vessels and fisheries) and into six categories related to activity of its origin (recreation, domestic, sanitary, industrial, fishing, aquaculture) (Koutsodendris et al., 2008). The category “mix” was added to both sub-classification since many litter items (e.g. sheets, plastic bottles and bags, etc.) have an uncertain source and may come from multiple activities (Table 3.3.2). The number of items and the weight of each litter category found in each haul were standardized to the square kilometer on the basis of the swept area. Since litter was recorded only from RAPIDO D, the total amount of debris for each haul is computed multiplying the litter sample weight/number with a raising factor (RF) defined as follow (SoleMon, 2012):

$$RF = (TOT\text{-}A + TOT\text{-}D) * (TOT\text{-}D)^{-1}$$

where TOT-A is the total weight of the catch of RAPIDO A and TOT-D is the total weight of RAPIDO D.

Table 3.3.2. Main pollution sources of the study areas based on the identification of litter items.

Material	Litter category	Source	Activities
Plastic	Bottles	Mix	Recreation
	Sheets	Mix	Mix
	Bags	Mix	Domestic
	Caps/lids	Mix	Recreation
	Mussel nets and ropes	Fisheries	Aquaculture
	Fishing lines (monofilament)	Fisheries	Fishing
	Fishing lines (entangled)	Fisheries	Fishing
	Synthetic ropes	Fisheries	Fishing
	Fishing nets	Fisheries	Fishing
	Cable ties	Fisheries	Fishing
	Strapping bands	Fisheries	Fishing
	Crates and containers	Land	Domestic
	Other plastic	Mix	Mix
	Diapers	Land	Sanitary
	Cotton buds	Land	Sanitary
	Cigarette butts	Vessels	Recreation
	Condoms	Land	Sanitary
	Syringes	Land	Sanitary
	Sanitary towels/ tampons	Land	Sanitary
	Other sanitary waste	Land	Sanitary
Metal	Cans (food)	Vessels	Recreation
	Cans (beverage)	Vessels	Recreation
	Fishing related	Fisheries	Fishing
	Drums	Vessels	Industrial
	Appliances	Vessels	Domestic
	Car parts	Land	Recreation
	Cables	Vessels	Industrial
	Other metals	Fisheries	Fishing
Rubber	Boots	Fisheries	Fishing
	Balloons	Land	Recreation
	Bobbins (fishing)	Fisheries	Fishing
	Tyres	Fisheries	Fishing
	Gloves	Fisheries	Fishing
	Other rubber	Mix	Mix
Glass	Jars	Vessels	Recreation
	Bottles	Vessels	Recreation
	Pieces	Vessels	Recreation
	Other glass/ceramic	Vessels	Mix
Natural	Wood (processed)	Land	Industrial
	Ropes	Fisheries	Fishing
	Paper/Cardboard	Vessels	Recreation
	Pallets	Vessels	Industrial
Mix	Clothing/ rags	Mix	Mix
	Shoes	Vessels	Domestic
	Other miscellaneous	Mix	Mix

3.3.3 Identification of sources

Since one of the aims of the present study was to identify the role of different litter sources on quali-quantitative spatial distribution of seafloor debris, GIS layers regarding potential litter sources (rivers, cities, shipping lanes and mussel farms) in the Adriatic were collected. Rivers and cities databases were retrieved from Geofabrik's free download server (http://download.geofabrik.de/europe.html). Rivers with an average discharge > 200 m^3/sec were taken into consideration: Po, Adige and Piave rivers in Italy, and Neretva river in Croatia. Nine largest cities (> 90,000 inhabitants) located within 10 km from the coastline were considered: Trieste, Venice, Rimini, Pesaro, Ancona, Pescara, Bari along the Italian coast, and Split and Rijeka in Croatia. Regarding shipping lanes, the annually averaged traffic density map provided by Automatic Identification System (AIS, 2015) was used in order to identify most congested lanes. Each lane is described by the geographical coordinates of the two end-points (longitudes and latitudes) and the average traffic intensity, expressed using a three-levels scale (low, moderate, high). As for litter inputs from aquaculture, a map of Adriatic mussel farms was retrieved from http://lizmap.arpa.fvg.it/. Since some of the sources detected (Neretva river, Split, Rijeka and Bari) were not included in the study area, they were not considered in the analyses (Fig. 3.3.4). The distance from the closest litter sources was computed for each haul obtaining the continuous explanatory variables for multivariate analysis. Data were analyzed using ESRI® ArcGIS 10.1.

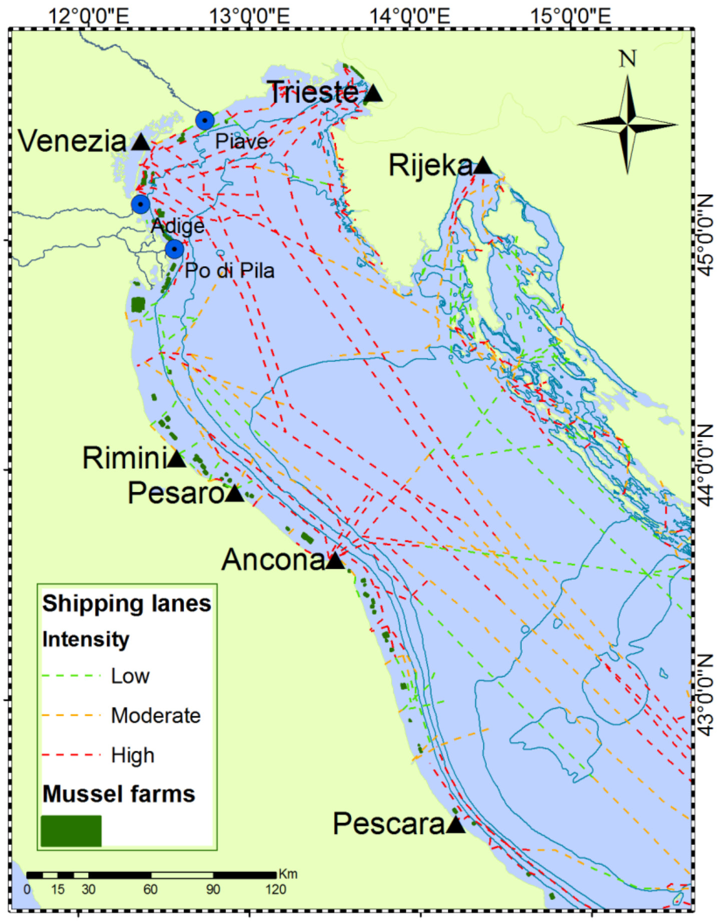


Figure 3.3.6. Spatial distribution of marine litter inputs into the Adriatic basin: shipping lanes; mussel farms; largest rivers (average annual discharge > 200 m3/sec; labeled closed blue circles); largest cities (population > 90,000; labeled closed black triangles).

3.3.4 Data analysis

The relationship among explanatory variables was investigated through linear regression analysis to identify potential cases of collinearity (Zuur et al., 2007) and consequently reduce the number of variables for the analyses. Relationships between marine litter densities (response variables) and sources (explanatory variables) were investigated through Canonical Correspondence Analysis (CCA). CCA is a multivariate technique based on unimodal relationships between response and environmental variables, similar to Redundancy Analysis (RDA), except that the Chi-square distance function is used as the measure of association instead of covariance/correlation coefficients. In CCA, the sum of all canonical eigenvalues is used as a tool to assess how well a specific selection of explanatory variables explains the variance in the litter data (Zuur et al., 2007). We tested the significance of the CCA axes by running 999 unrestricted permutations in Monte Carlo tests, using the axes' eigenvalues as the test statistics. To interpret the ordination axes the correlation coefficients between each explanatory variable and each ordination axis was calculated. By looking at the signs and relative magnitudes of these correlation coefficients we could infer the relative importance of each litter source for predicting the litter composition (Ter Braak and Prentice, 1988). The original litter data set contained densities of 45 litter categories, most of them were rare. To reduce the large number of double-zeros in the data matrix and to better explain results in terms of activities that may generate marine litter, density data were grouped by activity of origin and CCA was done on aggregated data (Table 3.3.2). The analysis was performed using PAST software (Hammer et al., 2001).

Differences in litter composition were further investigated through the non-parametric multivariate permutational analysis of variance (PERMANOVA; Anderson et al., 2008) according to a one-way experimental design. This non-parametric test was used in order to avoid problems with the asymmetrical distribution of the data and to maximize the robustness of results. The resemblance matrix was based on Bray-Curtis similarity index. Two different analyses were performed. In the first case, hauls were grouped by depth stratum (factors) and density data grouped by activity of origin were used (Table 3.3.3). In the second case, hauls were grouped by source (factors) which affected the litter composition most (according to CCA results) and raw density data were used. An uncorrected P-value ($\alpha = 0.05$) was provided performing 9999 permutation of untransformed data.

The Similarity Percentages (SIMPER) analysis using the Bray-Curtis similarity measure was used when results from PERMANOVA were significant to identify litter items aggregated by activity of origin and/or original litter categories that were most important in defining differences between factors (Clarke, 1993). The analysis was performed using PAST software (Hammer et al., 2001).

Statistical analyses were based on density data expressed in number of items/square kilometer. The use of abundance instead of weight was preferred in order to give more emphasis to the nature/category of litter items rather than their mass (Galgani et al., 2013). Moreover, litter type with different weights (e.g. heavy metal objects vs. light plastic) cannot be compared (Koutsodendris et al., 2008) and certain litter debris have a high wet-weight (e.g. clothes, shoes, processed wood) which leads to overestimate their abundances.

3.4 Macrolitter ingested by fish

3.4.1 Sample collection

Fish samples were collected from November 2014 to August 2015 during DeFishGear and SoleMon trawl-surveys. The sampling area was located in the Western Gulf of Venice (GSA17) between Lignano Sabbiadoro (UD) and the River Po mouth (Fig. 3.4.2), characterized by sandy and soft bottoms. This area was chosen since it represents an important fishing ground (Mion et al., 2015) concentrating more than 15% of the Italian fishing activities, making it the most exploited Italian basin (Pranovi et al., 2015).

DeFishGear surveys were performed with the mid-sized commercial fishing boats "Jolly" (length: 17 m; tonnage: 14 TJB; power: 149 kW) and "Drago" (length: 18 m; tonnage: 9.53 TJB, power: 180 kW), captained by local fishermen of Chioggia. Vessels were equipped with a bottom otter trawl (square-mesh size: 40 mm), a cone-shaped net consisting of a body closed by one or two codends, with lateral wings extending forward from the opening (Fig. 3.3.1). The net is kept open horizontally (horizontal opening: 10 m) by two otter boards, vertically (vertical opening: 1 m) by floats on the upper edge and weights on the groundrope. The trawl usually targets benthic and demersal species, tough the net can be towed at different distances from the bottom, adding more or less weighted groundrope, allowing to catch also mesopelagic and pelagic species.

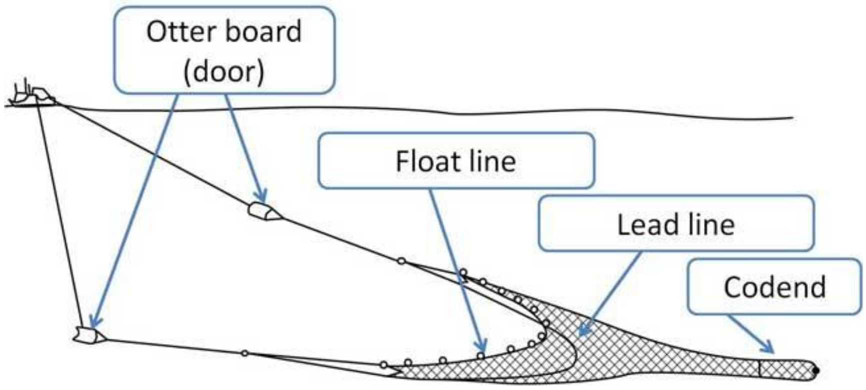


Figure 3.4.1. Structure of a bottom otter trawl used by Chioggia's fishing fleet.

Fish samples were collected at 16 stations (Fig. 3.4.2) in Autumn 2014 (first DeFishGear survey, DFG1). Trawls were performed at a mean speed of 2.4 ± 0.1 knots and at a depth ranging between 10 and 31 m. Hauls were made during daylight and lasted approximately 30 minutes, starting when the gear settled on the bottom and ending when hauling commenced. Additional fish samples were taken from eight stations during the second DeFishGear survey (DFG2, June 2015) and from three stations during the third DeFishGear survey (DFG3, August 2015) (Fig. 3.4.2). Additional samples came from 4 stations (Fig. 3.4.2) of the SoleMon trawl survey (Autumn 2014), which was performed with the research vessel "G. Dallaporta", equipped with the *rapido* trawl. Fishing gear features and hauls information had already been described in chapter 3.3.1.

After capture, fish samples were frozen immediately and transported to the Biology Laboratory of the branch office of Chioggia of the "Istituto Superiore per la Protezione e la Ricerca Ambientale" (ISPRA). On board, the following information were recorded: fishing location and haul number, date of capture, sampling gear, day time and depth.

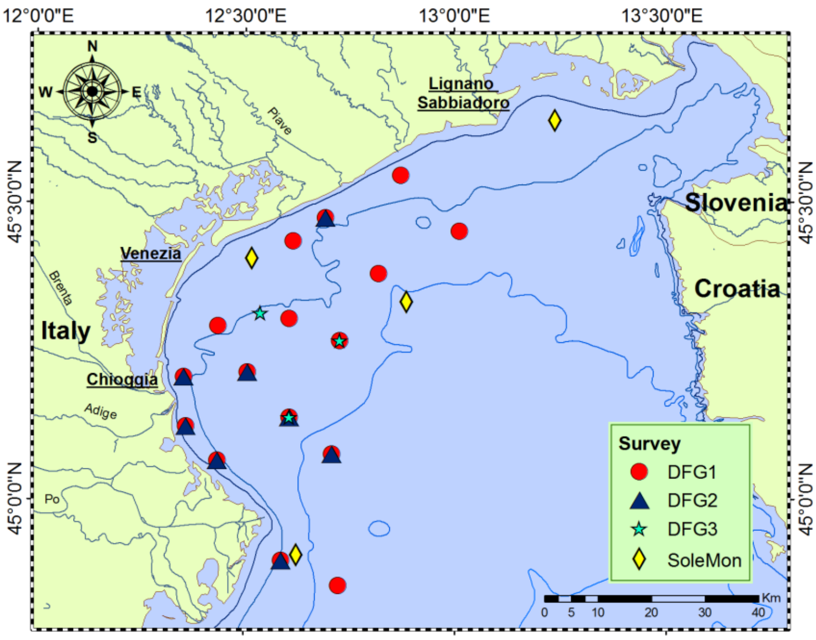


Figure 3.4.2. Location of the sampling stations in the Western Gulf of Venice. **DFG1**: first DeFishGear survey (Autumn 2014); **DFG2**: second DeFishGear survey (June 2015); **DFG3**: third DeFishGear survey (August 2015).

3.4.2 Laboratory analysis

For the purpose of the study, the following species were selected as target: *Mullus barbatus* and *M. surmuletus* (Red mullet and Striped red mullet), *Chelidonichthys lucerna* (Tub gurnard), *Solea solea* (Common sole), *Pagellus erythrinus* (Common pandora), *Sparus aurata* (Gilthead seabream), *Mustelus* spp. (Smooth-hounds) and *Sardina pilchardus* (European pilchard).

S. *solea*, *M*. *barbatus*, *M. surmuletus* and *C. lucerna* are demersal species living on sand, muddy-sand or gravel bottoms and feed on fish, crustaceans and mollusks during daytime (Ben-Tuvia**,** 1990), while *S. solea* has nocturnal feeding habits. *P. erythrinus*, *S. aurata* and *Mustelus* spp. are benthopelagic species, inhabiting the water just above the bottom. The first two species belong to the family of Sparidae, they are omnivorous, but feed mainly on benthic invertebrates, shellfish and small fishes (Bauchot and Hureau, 1990). *Mustelus* spp. (*M. mustelus*, *M. punctulatus* and the hybrid were pooled together because of the lack of unambiguous morphological traits for their identification; Barausse et al., 2014) are carnivorous species, feeding mainly on crustaceans, but also cephalopods and bony fishes (Compagno, 1984). *S. pilchardus* is an important target species for fishery in the Adriatic region. It inhabits the pelagic domain, where it forms schools and feeds mainly on planktonic crustaceans during night time (Brito, 1991).

In the laboratory, samples were analyzed according to the protocol defined in the framework of DeFishGear project (Anastasopoulou and Mytilineou, 2015). Each individual was given an ID number, and main biological parameters were recorded: length, weight, sex and maturity stage when possible (Fig. 3.4.3 A-B). Afterwards, fish were dissected carefully to avoid cutting internal organs. The gastro-intestinal tract was removed and placed in a sealed bag on which the fish ID was annotated, and it was frozen again (Fig. 3.4.3 C-D). In order to avoid external airborne contamination, workbench was carefully cleaned, air conditioning was turned off, and gloves and white coat were used.

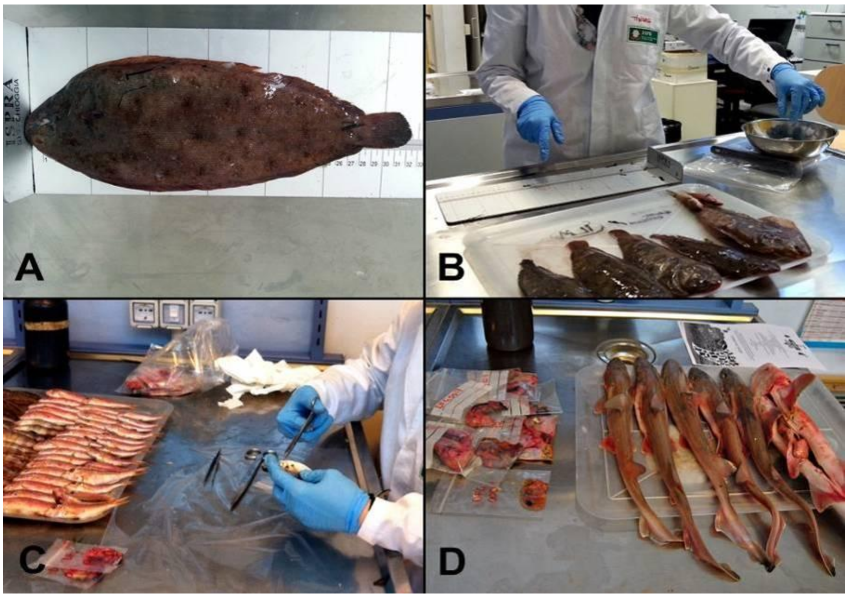


Figure 3.4.3. Fish samples processing for the gastro-intestinal content analyses.

During analyses, stomach and intestine contents were extracted, placed separately in a petri-dish and weighted (Fig. 3.4.4 A-D). A fullness index was assigned, using an empirical 6-stage scale with 0 as empty and 5 as very full stomach. Contents were subsequently examined using a binocular Leica MS5 stereomicroscope (from 6.3× to 40× magnification). Any anthropogenic object bigger than 1 mm in the longest dimension was classified according to the Master list of categories of litter proposed by MSFD TSG10 Report (Table 3.4.1). The following parameters were also recorded: location (Stomach/Intestine), shape, size (using a millimetric graph paper) and color.

Table 3.4.1. Master list of Categories of Macro Litter Items.

General Code	General Name	Materials
G112	Industrial pellets	Artificial polymer materials
G118	Small industrial spheres (<5mm)	Artificial polymer materials
G119	Sheet like user plastic (>1mm)	Artificial polymer materials
G120	Threadlike user plastic (>1mm)	Artificial polymer materials
G121	Foamed user plastic (>1mm)	Artificial polymer materials
G122	Plastic fragments (>1mm)	Artificial polymer materials
G157	Paper	Paper/ Cardboard
G183	Fish hook remain	Metal
G212	Slack/Coal	
G213	Paraffin/Wax	Chemicals
G214	Oil/Tar	Chemicals
G215	Food waste (galley waste)	Organic
G216	Various rubbish (worked wood, metal parts)	Unidentified
G217	Other (glass, metal, tar) < 5 mm	Unidentified

Since during laboratory processing environmental contamination of samples by airborne microfibers could occur, the DeFishGear protocol (Anastasopoulou and Mytilineou, 2015) suggested to place a clean petri-dish near the working area. This petri-dish, used as blank sample, was examined under the stereomicroscope at the beginning of daily laboratory activity and cleaned from possible fibers. At the end of the day, the blank sample was examined again, and fibers on it were classified and measured. Fibers with the same characteristics in terms of shape and color that were found either in the blank sample and in gut contents were excluded from analyses, following the methodology described by Davison and Asch (2011).

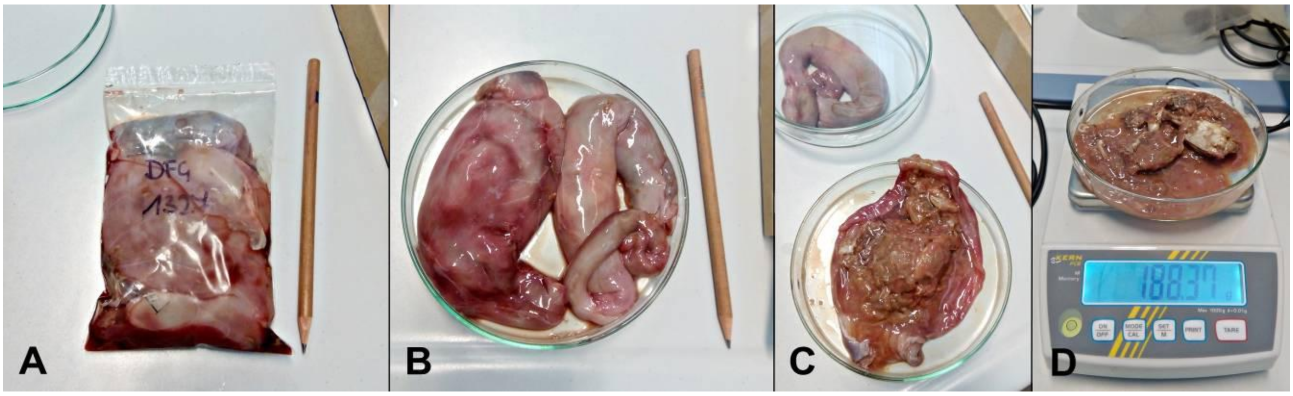


Figure 3.4.4. Gut content examination process in the biology laboratory.

3.4.3 Data analysis

For litter analysis the following indexes were used:

1. The vacuity index (VI) = [(number of empty gut or stomachs or intestines/number of gut or stomachs or intestines examined) x 100].
2. The percentage frequency of occurrence (%F) = the number of gut or stomachs or intestines containing a given litter item/total number of non-empty gut/stomachs/intestines examined x 100.
3. The percentage numerical abundance (%N) = the number of litter items of a given litter category in all non-empty gut or stomachs or intestines/total number of litter items of all categories in all stomachs/intestines x 100.

4. RESULTS

4.1 Beach litter monitoring

4.1.1 Litter abundance

7,301 items corresponding to 132.23 Kg of litter were totally collected in the three sites during the spring survey, and 4,172 items, corresponding to 58.41 Kg, during the summer survey, for a total amount of 11,473 items and 190.64 Kg. Items ranged in size from large fishery supplies, as fenders and buoys, to small fragments of hard plastic and sheet.

The visual appearance of the beaches in spring was quite bad, as several storms occurred during winter, leading the litter to be stranded on the shore, especially along the accumulation line at the base of the dune/vegetation (Fig. 4.1.1). The Clean Coast Index classified Rosolina mare as a ''Dirty" beach (CCI = 13.7); the other beaches ranked even as ''Extremely dirty": Boccasette, CCI = 36.3; Torre del Cerrano, CCI = 28.4. Overall, Boccasette was the most polluted beach contributing with the 45% of waste collected in terms of number and with the 41% of the total weight, followed by Torre del Cerrano (36% in terms of number and 21% of the total weight) and Rosolina mare (20% in terms of number and 38% of the total weight). The mean litter density at Boccasette was 1.8 ± 0.3 items/m^2, corresponding to 32 ± 16 g/m^2. In Torre del Cerrano the mean density of litter was 2 ± 1 items/m^2 (15 ± 10 g/m^2) and in Rosolina mare the mean density of litter was 0.69 ± 0.03 items/m^2 (25 ± 11 g/m^2) (Fig. 4.1.2).

Figure 4.1.1. Litter accumulation line at Boccasette beach during the spring survey.

During the summer survey, the visual appearance of the beaches was generally better than in the spring, except for Boccasette, where large amounts of litter were found at the back of the beach, close to the dunes. Indeed, the Clean Coast Index classified Boccasette again as a ‘‘Extremely dirty” beach (CCI = 25.2), while the other beaches ranked as ‘‘Moderate”: Rosolina mare, CCI = 9.6; Torre del Cerrano, CCI = 9.9. As shown in Fig. 4.1.2, among sites there is a consistent decreasing trend in litter density from spring to summer season. However, Boccasette in summer still showed the highest values of density (1.2 ± 0.4 items/m^2; 19.6 ± 0.6 g/m^2), contributing with the 56% in terms of number of items and 63% of the total weight of waste collected. At Torre del Cerrano litter density in summer was three time less than in spring: 0.6 ± 0.3 items/m^2 density, corresponding to 7 ± 3 g/m^2. At Rosolina mare litter density in summer was 0.48 ± 0.03 items/m^2, corresponding to 19.6 ± 0.6 g/m^2.

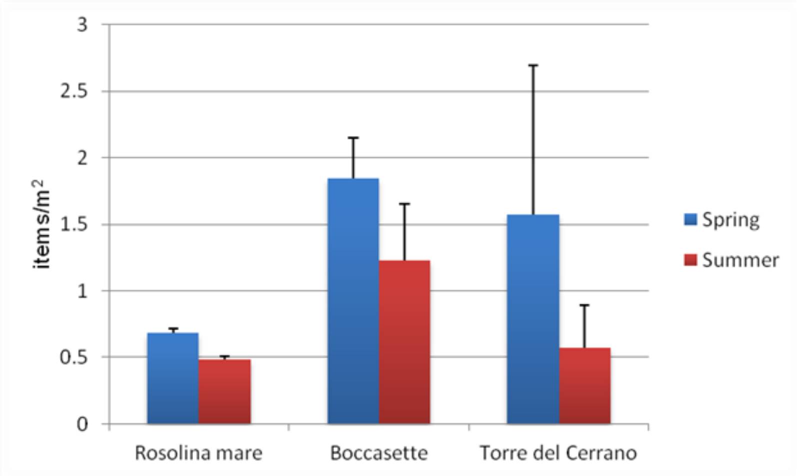
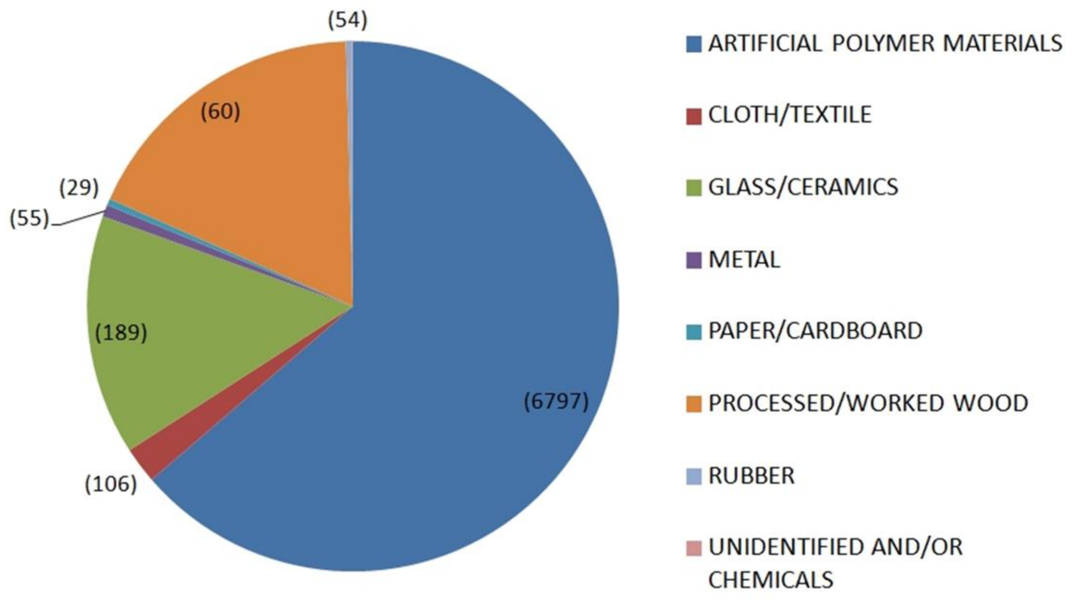


Figure 4.1.2. Density, in terms of numbers of items per square meter (items/m^2), of marine litter found in the three sites during the spring and summer surveys. Bars represent Standard Error.

4.1.2 Litter composition

In all surveys, the greater majority of debris was made of plastic (Fig. 4.1.3).

During the spring surveys, plastic represented almost the totality in terms of numbers (93%) and the majority in terms of weight (64%). Glass/ceramics was the second most abundant group (3% in terms of numbers, 15% in terms of weight), followed by cloth/textile (1.5% in terms of items, corresponding to 2% of total weight) (Fig. 4.1.3). The highest density of plastic was found in Boccasette, reaching a mean of almost 1.8 ± 0.3 items/m^2, followed by Torre del Cerrano (mean of 1 ± 1 items/ m^2) and Rosolina mare (mean of 0.64 ± 0.03 items/m^2) (Fig. 4.1.3).



Site	Area surveyed (m^2)	Artificial polymer materials	Cloth/ textile	Glass/ ceramics	Metal	Paper	Processed wood	Rubber	Other
Rosolina mare	2090	0.64 ± 0.03	< 0.01	0.011 ± 0.001	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Boccasette	1800	1.8 ± 0.3	0.017 ± 0.005	0.02 ± 0.01	< 0.01	< 0.01	0.017± 0.004	0.02 ± 0.01	< 0.01
Torre del Cerrano	1830	1 ± 1	0.04 ± 0.02	0.070 ± 0.001	0.015 ± 0.009	< 0.01	< 0.01	< 0.01	0

Figure 4.1.3. Marine litter composition in terms of weight found in the three sites during the spring survey. Numbers in parenthesis represent the total number of items found per each main group. The percentage of unidentified/chemicals items found (0.05%, corresponding to 11 items) was too low to enter the graph. Mean density of the main marine litter groups found in the three sites during the spring survey are shown in the table below. Numbers are expressed in terms of items/m^2 (± SE).

Plastic debris was generally composed by sheets derived from packaging (16%), mussel nets (13%), fragments (12%), polystyrene pieces (12%), cotton bud sticks (11%), caps and lids (8%) and bottles (4%) (Fig. 4.4.4). The abundance of categories varies among sites, but generally Boccasette had the highest values, especially regarding mussel nets (0.30 ± 0.01 items/ m^2), polystyrene pieces (0.24 ± 0.01 items/ m^2) and cotton bud sticks (0.219 ± 0.002 items/ m^2) (Fig. 4.1.5). Differences in litter composition among sites during the spring survey were not statistically significant (Table 4.1.1).

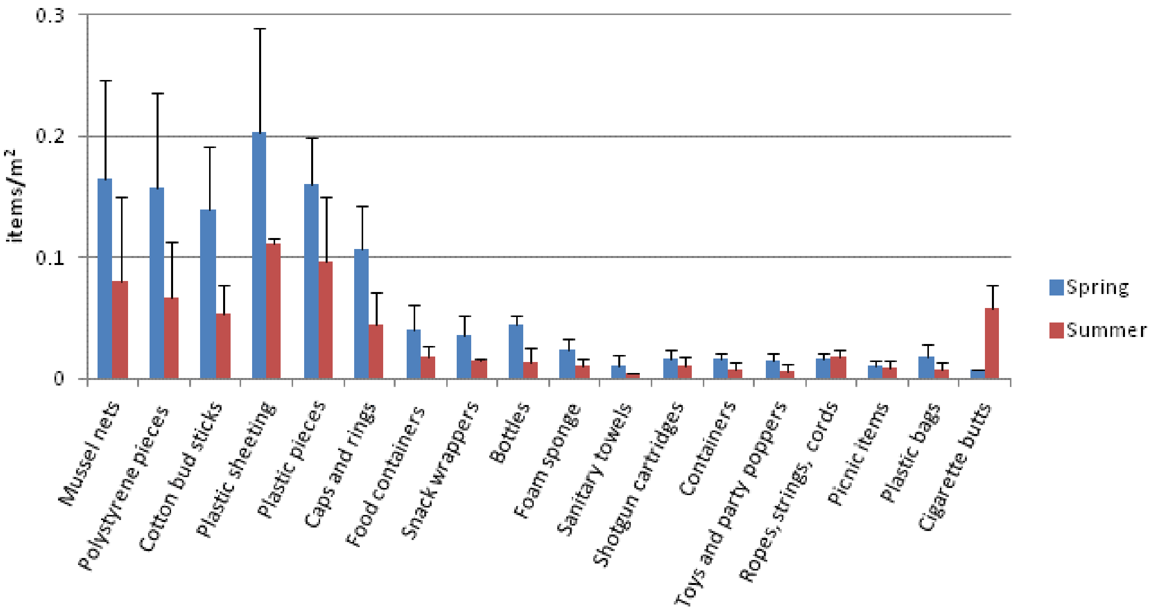


Figure 4.1.4. Mean density of eighteen main categories of plastic debris totally found during the spring and summer surveys. Bars represent Standard Error.

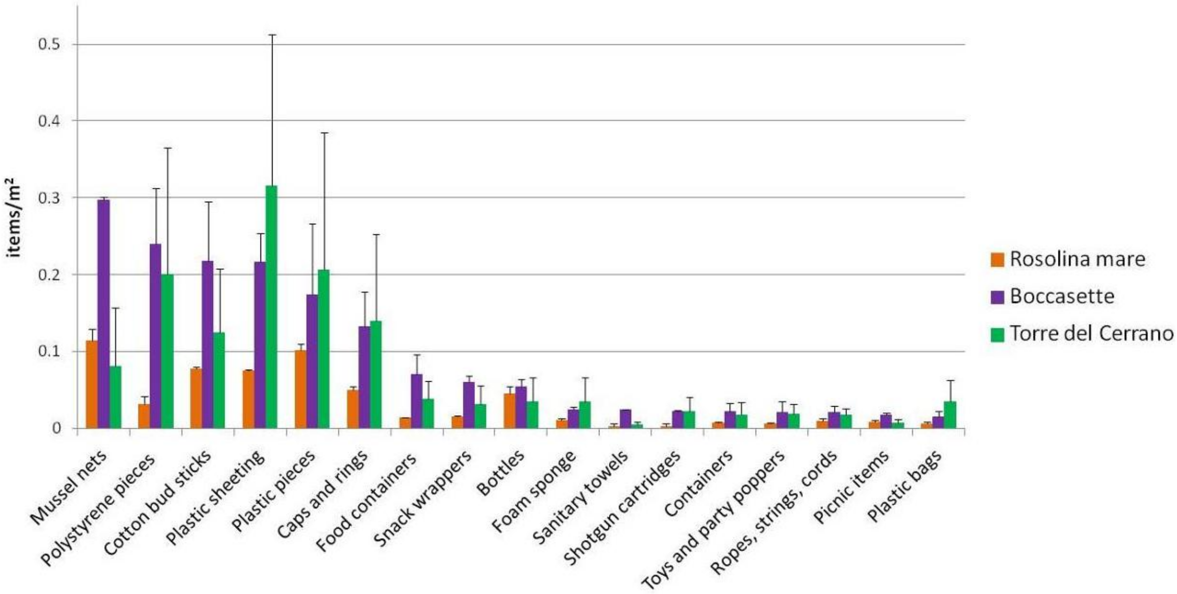
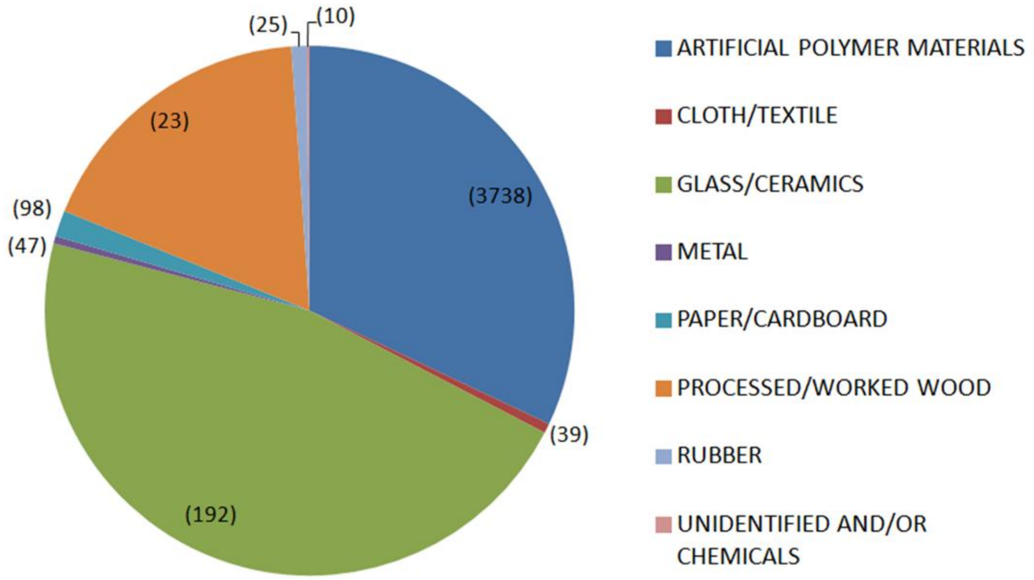


Figure 4.1.5. Mean distribution of seventeen main categories of plastic found in the three sites during the spring survey. Bars represent Standard Error.

Table 4.1.1. PERMANOVA analysis results basing on original litter categories. P-values were obtained using Monte Carlo samples (MC: Monte Carlo test) from the asymptotic permutation distribution .

	df	Total SS	Within-group SS	Pseudo-F	P	Unique perms
Litter composition						
Spring	2	0.6704	0.3233	1.6108	0.2668 (MC)	15
Summer	2	0.7544	0.3641	1.6077	0.2321 (MC)	15

In summer, the number and weight of debris collected during the survey decreased, but plastic still represented almost the totality in terms of numbers (90%) and a great percentage of the weight (32%), followed by glass/ceramics (5% in terms of numbers, 46% in terms of weight) and paper (2.3% in terms of numbers, corresponding to 2% of total weight) (Fig. 4.1.6). The highest density of plastic was found in Boccasette, reaching a mean of 1.2 ± 0.4 items/m^2, followed by Rosolina mare (mean of 0.44 ± 0.02 items/m^2) and Torre del Cerrano (mean of 0.4 ± 0.2 items/ m^2). Densities of the other groups are shown in Figure 4.1.6.



Site	Area surveyed (m^2)	Artificial polymer materials	Cloth/ textile	Glass/ ceramics	Metal	Paper	Processed wood	Rubber	Other
Rosolina mare	2090	0.44 ± 0.02	< 0.01	< 0.01	< 0.01	0.0124 ± 0.0004	< 0.01	< 0.01	< 0.01
Boccasette	1870	1.2 ± 0.4	< 0.01	< 0.01	0.02 ± 0.02	0.0203 ± 0.0004	< 0.01	< 0.01	< 0.01
Torre del Cerrano	1650	0.4 ± 0.2	0.01 ± 0.01	0.070 ± 0.001	< 0.01	0.020 ± 0.001	< 0.01	< 0.01	0

Figura 4.1.6. Marine litter composition in terms of weight found in the three sites during the summer survey. Numbers in parenthesis represent the total number of items found per each main group. Mean density of the main marine litter groups found in the three sites during the summer survey are shown in the table below. Numbers are expressed in terms of items/m^2 (± Standard Error).

In summer the majority of plastic was composed by sheets (16%), fragments (15%), mussel nets (12%), polystyrene pieces (10%), cotton bud sticks (8%) and caps and lids (6%). Cigarette butts represent a remarkable percentage (8%) in summer, increasing from a mean density of 0.0074 ± 0.0005 items/m^2 during spring, to 0.06 ± 0.01 items/m^2 (Fig. 4.1.4), with Torre del Cerrano having the highest density (0.08 ±

0.04 items/m^2) (Fig. 4.1.7). Even during summer season, no significant differences among sites were detected (Table 4.1.1).

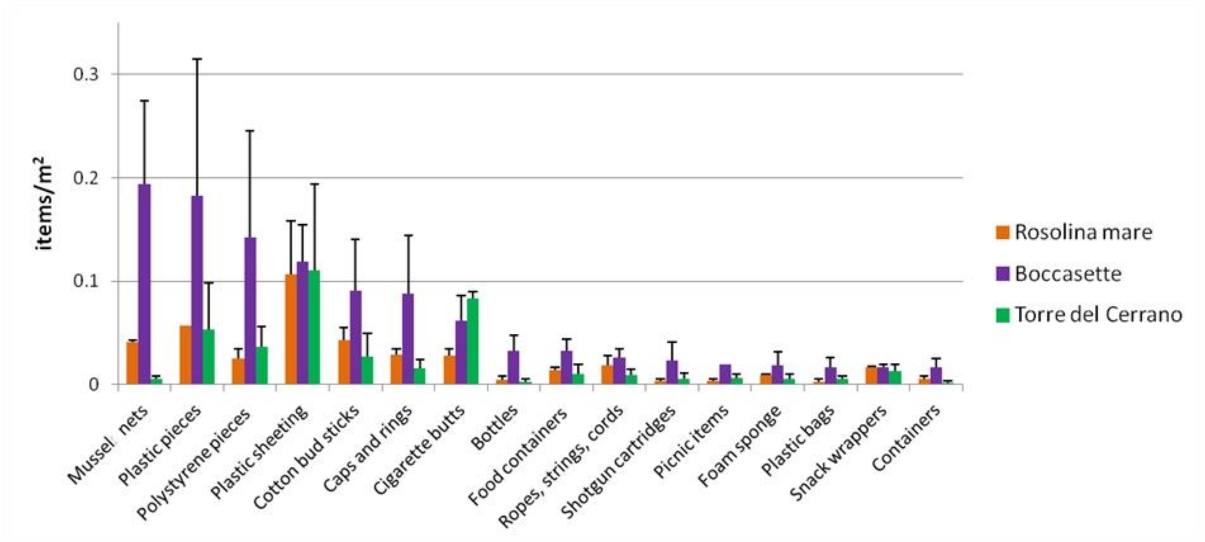


Figure 4.1.7. Mean distribution of seventeen main categories of plastic found in the three sites during the summer survey. Bars represent Standard Error.

4.1.3 Litter origin

Marine litter sources (Fig. 4.1.8) were primarily uncertain (46% in spring, 44% in summer), due to the high density of plastic sheets and fragments which may be originated by different activities. During spring, the second most abundant source was shoreline and recreational activities (17.7%), followed by ocean activities (13%), medical supplies (12.8%) and dumping (8.5%). Relatively few products of smoking-related activities were found in the marine litter composition (0.03 ± 0.01 items/m^2), accounting for 2% (Fig. 4.1.8).

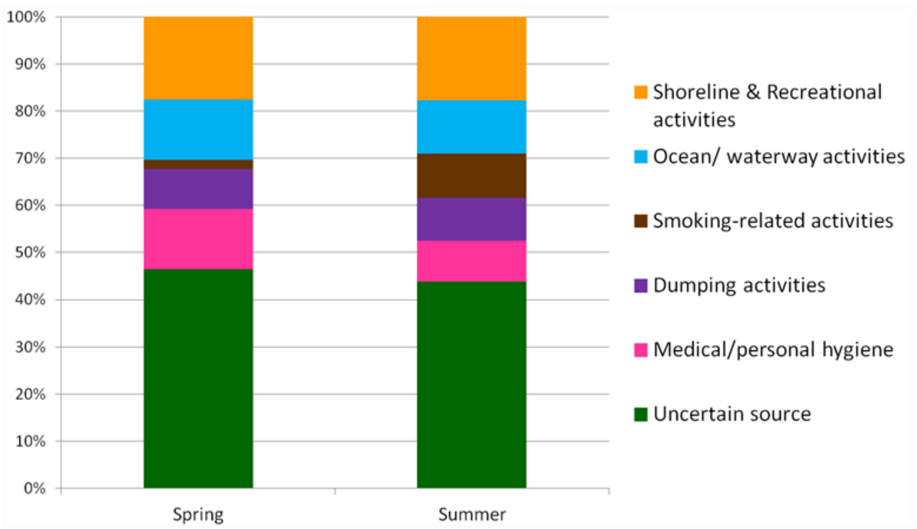


Figure 4.1.8. Sources of marine litter during the spring and summer surveys.

In summer, litter composition based on sources slightly changed: shoreline and recreational (18%) and ocean activities (11.4%) were followed by smoking-related products (9.3%), medical supplies (9%) and dumping (8.3%) (Fig. 4.1.8).

As shown in Figure 4.1.9, litter sources varied among sites and seasons and differences were tested through PERMANOVA. Results showed no significant differences among sites in spring neither in summer (Table 4.1.2). Differences among beaches were additionally tested through the Kruskal–Wallis test, analyzing sources individually for both seasons (Fig. 4.1.10 A-F). Significant differences were found for none of the six sources, neither in spring nor in summer.

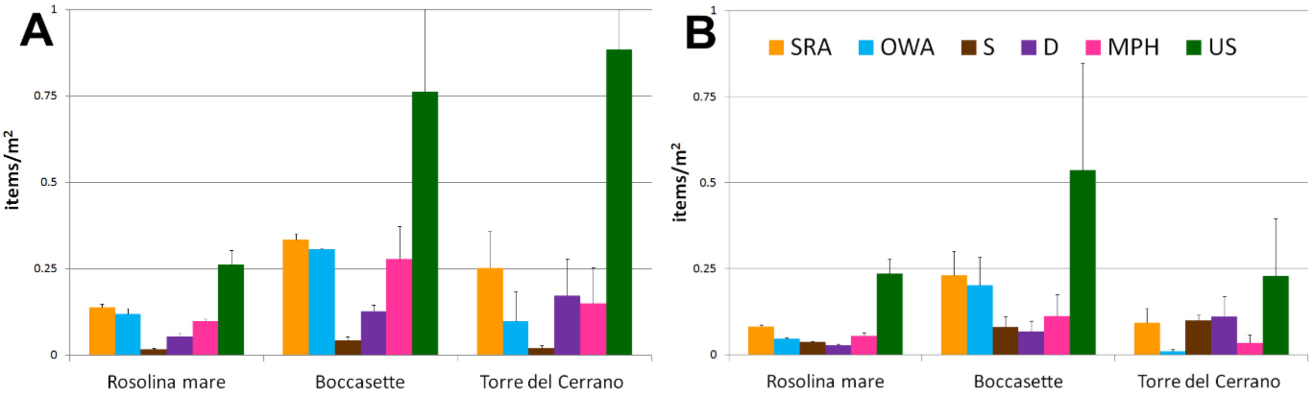


Figure 4.1.9. Mean density of marine litter aggregated by activity of origin (sources) found in the three sites during spring (**A**) and summer (**B**) surveys. (**SRA**: shoreline and recreational activities; **OWA**: ocean/waterway activities; **S**: smoking-related activities; **D**: dumping activities; **MPH**: medical/personal hygiene; **US**: uncertain source). Bars represent Standard Error.

Table 4.1.2. PERMANOVA analysis results basing on litter aggregated by activity of origin (sources). P-values were obtained using Monte Carlo samples (MC: Monte Carlo test) from the asymptotic permutation distribution.

	df	Total SS	Within-group SS	Pseudo-F	P	Unique perms
Sources						
Spring	2	0.5125	0.2723	1.3229	0.3712 (MC)	15
Summer	2	0.5125	0.2723	1.3229	0.3754 (MC)	15

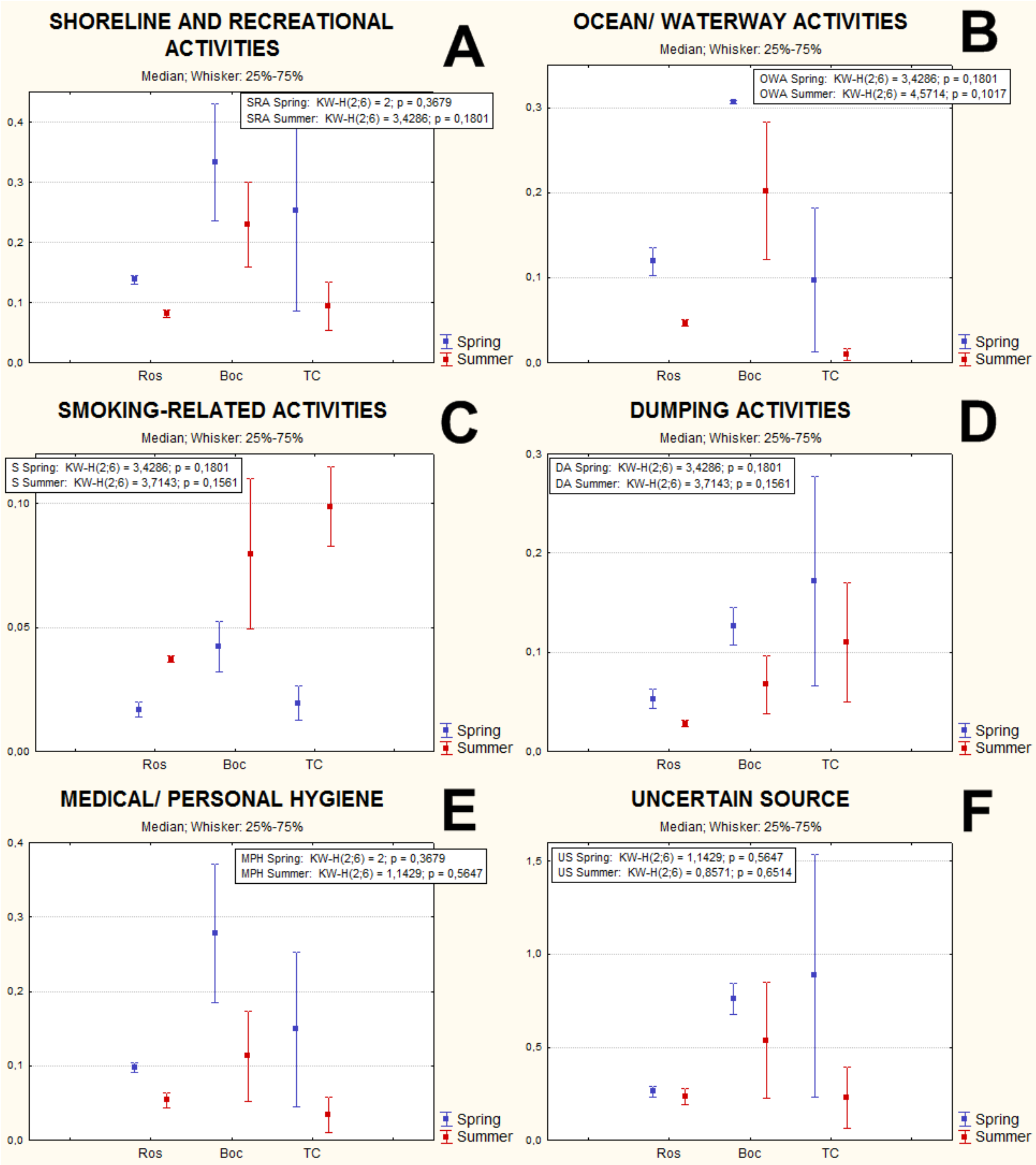


Figure 4.1.10. Box plots representing litter densities aggregated by activity of origin (source) by site for spring and summer season. Full squares represent median values. Results of Kruskal-Wallis test are shown in boxes above each graph. Ros = Rosolina mare; Boc = Boccasette; TC = Torre del Cerrano.

4.2 Benthic marine litter monitoring

4.2.1 Litter abundance and distribution

Overall, a total amount of 1013 items, corresponding to 83 kg of litter, were collected from RAPIDO D, for a total of 1.9 km^2 of seafloor surveyed. The mean number of items found in each haul was 15 ± 1, corresponding to 1.2 ± 0.6 Kg, whilst mean item weight was 223 ± 8 g.

The total mean density of litter recorded per haul was 913 ± 80 items/Km^2, corresponding to 82 ± 34 Kg/Km^2. The highest litter density was found in Stratum 1 (0-30 m), with a mean of 1128 ± 116 items/Km^2, corresponding to 116 ± 58 Kg/Km^2, followed by Stratum 3 (50-100 m, 892 ± 135 items/Km^2, 52 ± 21 Kg/Km^2) and Stratum 2 (30-50 m, 431 ± 62 items/Km^2, 22 ± 7 Kg/Km^2) (Fig. 4.2.1 A). Plastic was the dominant type of material in all strata (80% in terms of numbers and 62% in terms of weight), followed by glass and mixed material (Fig. 4.2.1 B-C).

The average densities of the six litter categories collected in the survey were: plastic 706 ± 72 items/Km^2 (49 ± 25 Kg/Km^2); glass 71 ± 18 items/Km^2 (4 ± 1 Kg/Km^2); mix 56 ± 18 items/Km^2 (14 ± 8 Kg/Km^2); metal 29 ± 7 items/Km^2 (9 ± 6 Kg/Km^2); natural 25 ± 7 items/Km^2 (4 ± 2 Kg/Km^2); rubber 25 ± 5 items/Km^2 (3 ± 1 Kg/Km^2).

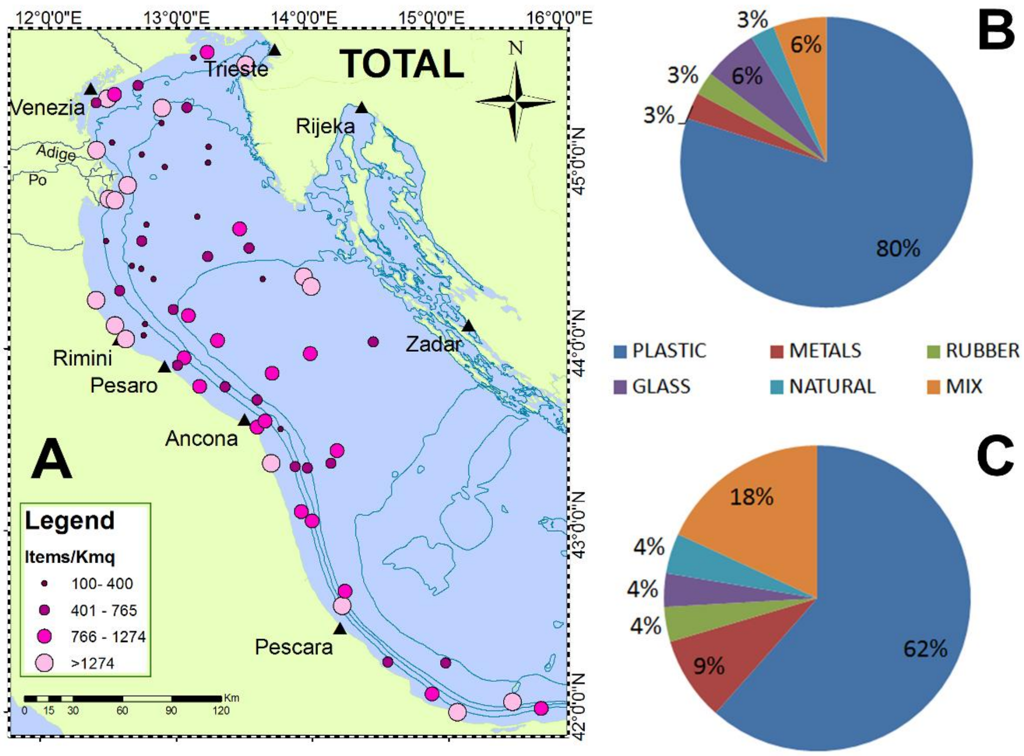
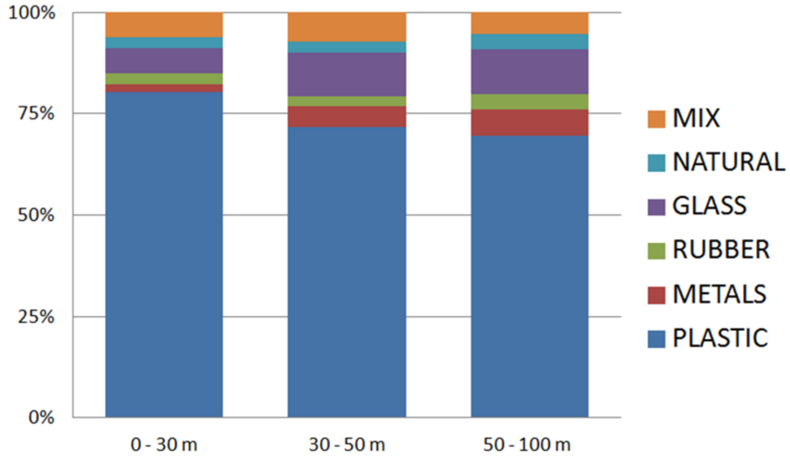


Figure 4.2.1. Spatial distribution of the total litter collected on the sea bottom during the SoleMon survey in 2014 (**A**) and total marine litter composition in terms of numbers (**B**) and weight (**C**).

Litter composition in the three strata was similar (Fig. 4.2.2), being plastic the most abundant material, followed by glass (jars, bottles, fragments) and mixed material. Densities of each material, grouped by depth stratum, are reported in Figure 4.2.2.



Depth	0 – 30 m		30 – 50 m		50 – 100 m	
	items/Km^2	SE	items/Km^2	se	items/Km^2	SE
Plastic	903	104	309	54	620	129
Metals	24	8	21	9	58	24
Rubber	28	7	10	7	33	15
Glass	72	27	47	29	100	27
Natural	30	10	11	8	33	28
Mix	69	29	32	11	48	43
Total	**1128**	**116**	**431**	**62**	**892**	**135**

Figure 4.2.2. Composition of marine litter in the three depth strata. Mean densities of the six litter categories found at different depths are shown in the table below. The mean density of the total litter is also reported. SE= Standard Error.

Plastic appeared widely distributed with the highest densities in the Northern Adriatic Sea, especially in front of the Po river estuary and touristic seaside cities as Rimini and Ravenna on the Northern coast, Pescara and Vasto on the Southern coast (Fig. 4.2.3 A). Metals and glass were more abundant in offshore stations (50–100 m), along major shipping routes connecting Northern Italy to Southern Italy and the Mediterranean countries (Fig. 4.2.3 B, D). Glass was abundant also in inshore stations located in front of main Northern Italian harbors, Venezia and Trieste. High densities of mixed material were found up to 30 m depth in front of largest cities of the Northern coast, and between 50 and 100 m depth, along the major shipping routes (Fig. 4.2.3 F). Natural (mainly worked or processed wood) was abundant along the Central coast, up to 30 m (Fig. 4.2.3. E), while rubber (balloons, tyres, fishing bobbins, boots and gloves) represented a poor category, heterogeneously distributed in the surveyed area (Fig. 4.2.3 C).

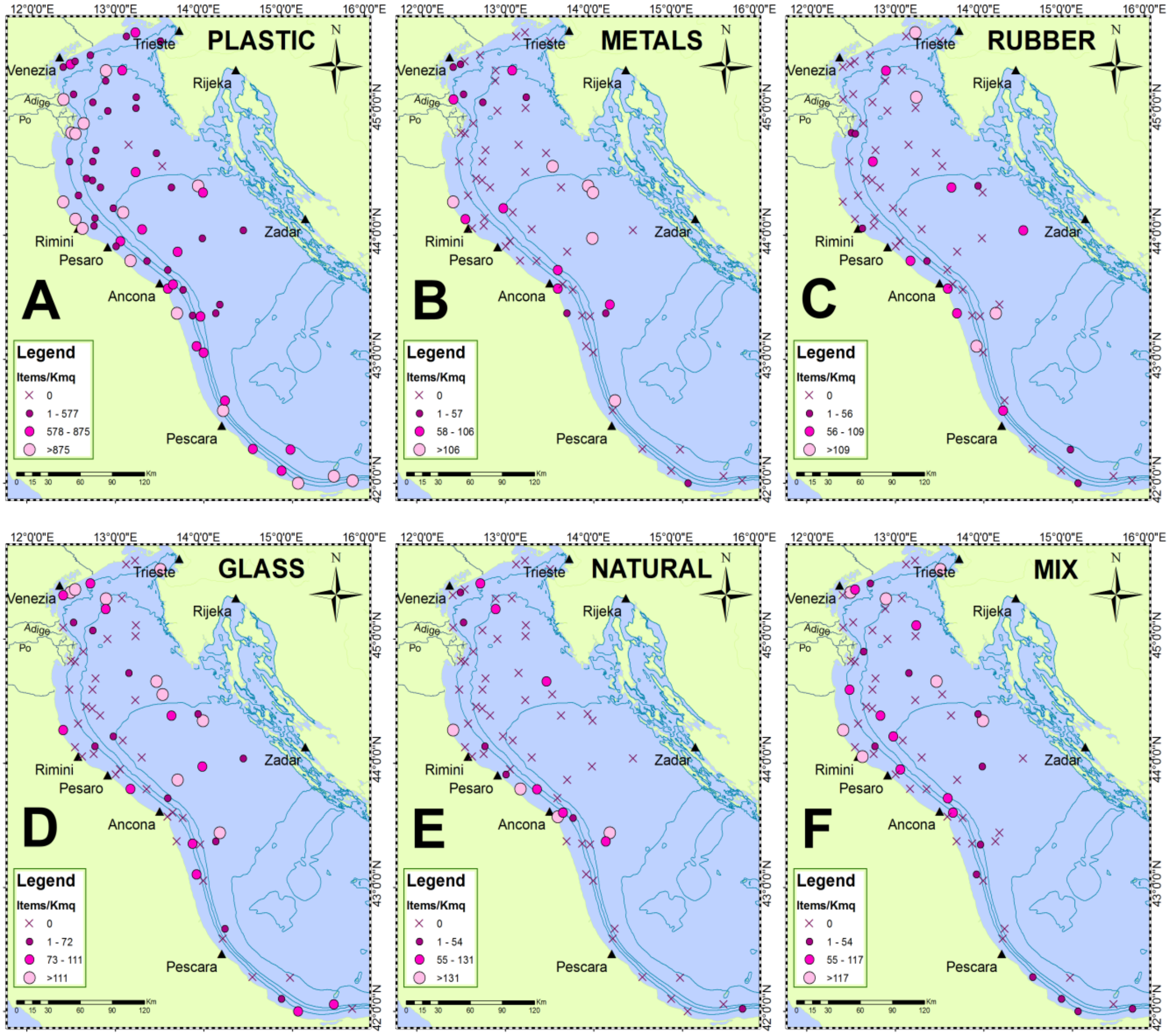


Figure 4.2.3 Spatial distribution of the six litter categories collected on the seafloor during the SoleMon survey in 2014.

4.2.2 Benthic litter composition and origin

In all strata plastic was mainly composed by bags (25%, 24% and 44%, respectively), sheets from packaging (23%, 36% and 19%, respectively) and mussel nets (24%, 9% and 4%, respectively) (Fig. 4.2.4). The distribution of categories varied among strata, but generally inshore stations (0-30 m) had the highest values, especially regarding mussel nets (217 ± 77 items/ Km^2) and sheets (209 ± 45 items/ Km^2).

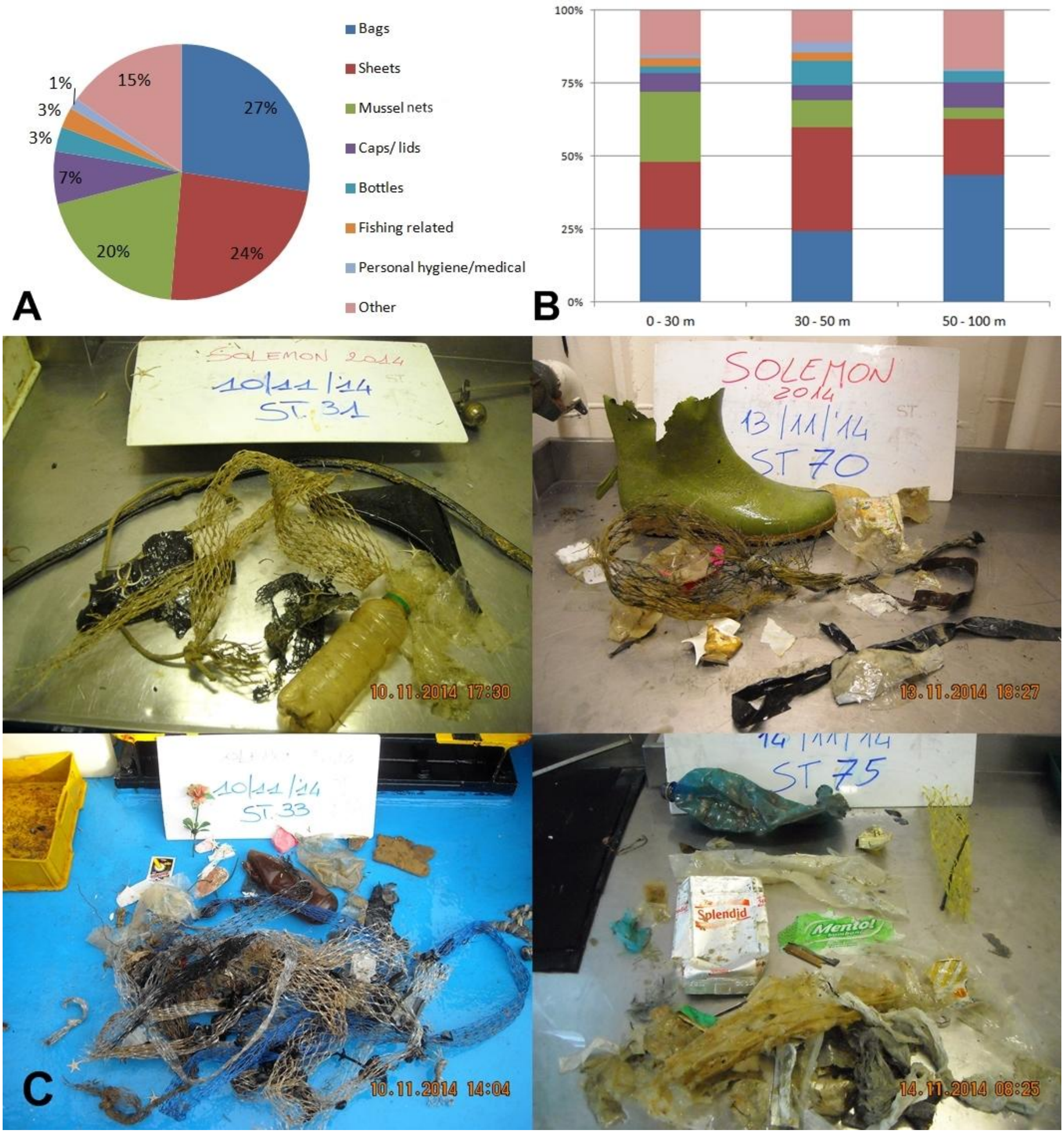


Figure 4.2.4. A: Composition of plastic waste collected during the SoleMon survey in 2014. **B**: Composition of plastic waste according to depth. **C:** Some of the litter categories collected from hauls during the monitoring.

For most of benthic marine litter it was not possible to univocally attribute a specific source (Fig. 4.2.5 A): mix source represented the major percentage (62%) of debris origin, followed by fisheries (22%), vessels (10%) and land (6%). 70% of litter items coming from fisheries (including aquaculture) was collected from stations up to 30 m depth, whilst offshore stations were the main source of litter from land (56%) and vessels (48%) (Fig. 4.2.5 B).

Even regarding the activity of origin, in most cases it was not possible to univocally attribute an item to a specific category (Fig. 4.2.6 A). The majority of waste collected originated from multiple activities (34%), followed by domestic (24%) and recreational (17%) activities. A sensible percentage of litter came from aquaculture activities (15%), in particular from coastal mussel farming: in fact, mussel nets were mainly collected from inshore stations (51% of the total) (Fig. 4.2.6 B).

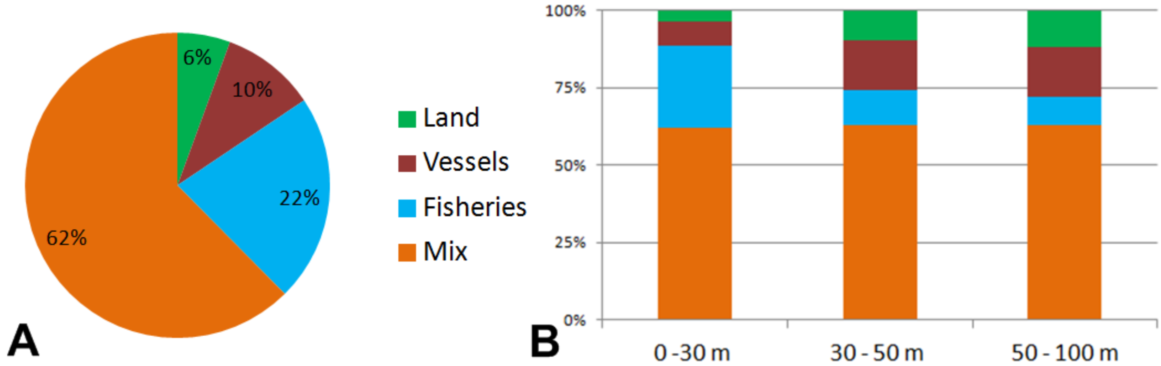


Figura 4.2.7. A: composition of benthic marine litter based on its source and **B**: distribution of litter source according to depth stratum.

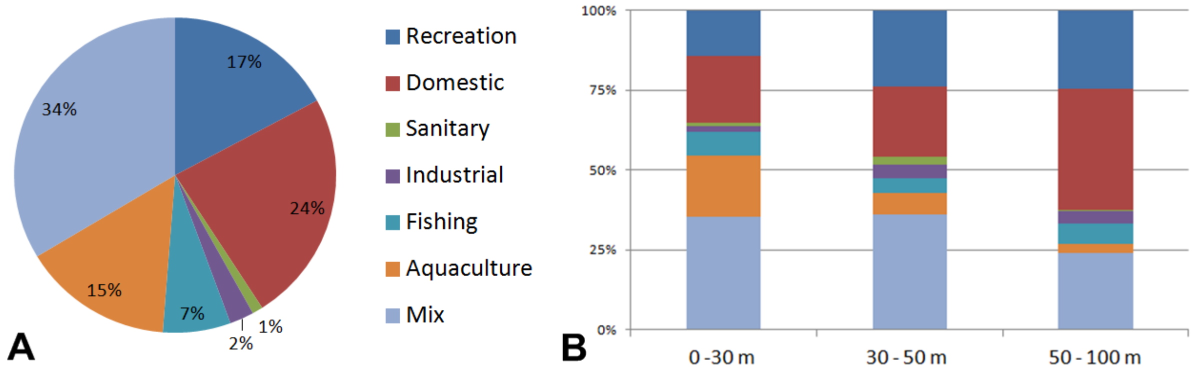


Figura 4.2.8. A: composition of benthic marine litter based on the activity of its origin and **B**: distribution of activity of litter origin according to depth stratum.

4.2.3 Multivariate analysis

Correlation analysis indicated serious collinearity (r > 0.95 or r < -0.95) between explanatory variables (Table 4.2.1 and Figure 4.2.7) and it was decided to omit from multivariate analysis some variables. Seven explanatory variables (litter sources) were ultimately chosen:

1. Po river;
2. Trieste;
3. Rimini;
4. Ancona;

5. Pescara;
6. Shipping lanes;
7. Mussel farms.

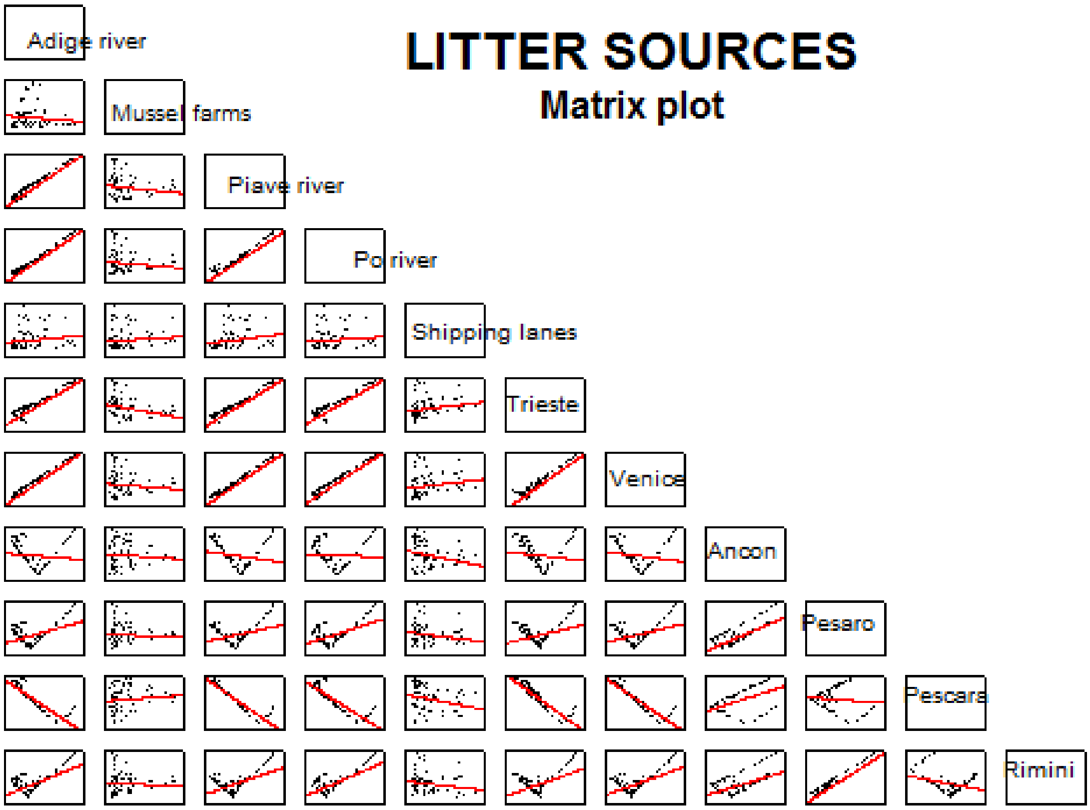


Figure 4.2.9. Matrix plot of explanatory variables.

Table 4.2.1. Correlation matrix between explanatory variables. Red marked correlations are significant at p < 0.05. Bold red marked numbers indicate strong correlation (r > 0.95 or r < -0.95) between variables.

	Adige river	Mussel farms	Piave river	Po river	Ship. lanes	Trieste	Venice	Ancona	Pesaro	Pescara	Rimini
Adige river	1,00	-0,11	**0,98**	**0,99**	0,14	0,94	**0,99**	-0,11	0,46	-0,89	0,67
Mussel farms	-0,11	1,00	-0,17	-0,13	0,10	-0,31	-0,12	-0,10	-0,09	0,11	-0,05
Piave river	**0,98**	-0,17	1,00	**0,96**	0,19	**0,97**	**1,00**	-0,19	0,36	-0,93	0,57
Po river	**0,99**	-0,13	**0,96**	1,00	0,10	0,92	**0,98**	-0,03	0,53	-0,85	0,73
Ship. lanes	0,14	0,10	0,19	0,10	1,00	0,19	0,17	-0,27	-0,21	-0,25	-0,19
Trieste	0,94	-0,31	**0,97**	0,92	0,19	1,00	0,95	-0,14	0,36	-0,89	0,54
Venice	**0,99**	-0,12	**1,00**	**0,98**	0,17	0,95	1,00	-0,17	0,39	-0,92	0,61
Ancona	-0,11	-0,10	-0,19	-0,03	-0,27	-0,14	-0,17	1,00	0,77	0,48	0,56
Pesaro	0,46	-0,09	0,36	0,53	-0,21	0,36	0,39	0,77	1,00	-0,09	**0,95**
Pescara	-0,89	0,11	-0,93	-0,85	-0,25	-0,89	-0,92	0,48	-0,09	1,00	-0,34
Rimini	0,67	-0,05	0,57	0,73	-0,19	0,54	0,61	0,56	**0,95**	-0,34	1,00

The eigenvalues of the first two CCA axis were 0.278 and 0.063, making 86.3% of the total variation explained. Both axes were significant ($p = 0.001$ and $p = 0.049$, respectively) in the Monte Carlo test with 999 permutations. CCA results show that offshore stations (from 30 to 100 m depth) were similar to each other in terms of litter composition according to the activity of origin, being poor in aquaculture and fishing related items and mainly composed by debris coming from domestic, recreational and mixed activities (Fig. 4.2.8). This result was confirmed by comparing stations scores, being stations close to each other similar in terms of Chi-square distances. Inshore stations (0–30 m depth) were heterogeneous in terms of litter composition and were those contributing most to the inertia (variation). As suggested by the CCA triplot (Figure 4.2.8), vicinity to shipping lanes and mussel farms has a strong effect on litter composition. Comparing litter categories scores and sources (explanatory variables) it is possible to infer that high density of aquaculture related debris occurred at low distance from mussel farms and high distances from shipping lanes. Indeed, as regards litter sources mussel farms and shipping lanes are the ones mostly negatively related (Figure 4.2.8). Projecting the lines on the axes it is possible to infer that the first axis is highly positively related with shipping lanes and negatively with mussel farms. Thus, the first CCA axis seems to reflect a gradient of vicinity to shipping lanes and mussel farms. The second axis is related with Trieste and Po river (positively) and Pescara and Ancona (negatively) (Fig. 4.2.8 and Table 4.2.2).

Table 4.2.2. Correlation coefficients between explanatory variables (litter sources) and the first two CCA axes.

Variables	Axis 1	Axis 2
Ancona	-0,05	-0,21
Pescara	0,00	-0,33
Rimini	-0,23	0,08
Mussel farms	-0,35	-0,20
Po river	-0,03	0,27
Shipping lanes	0,43	0,07
Trieste	0,11	0,35

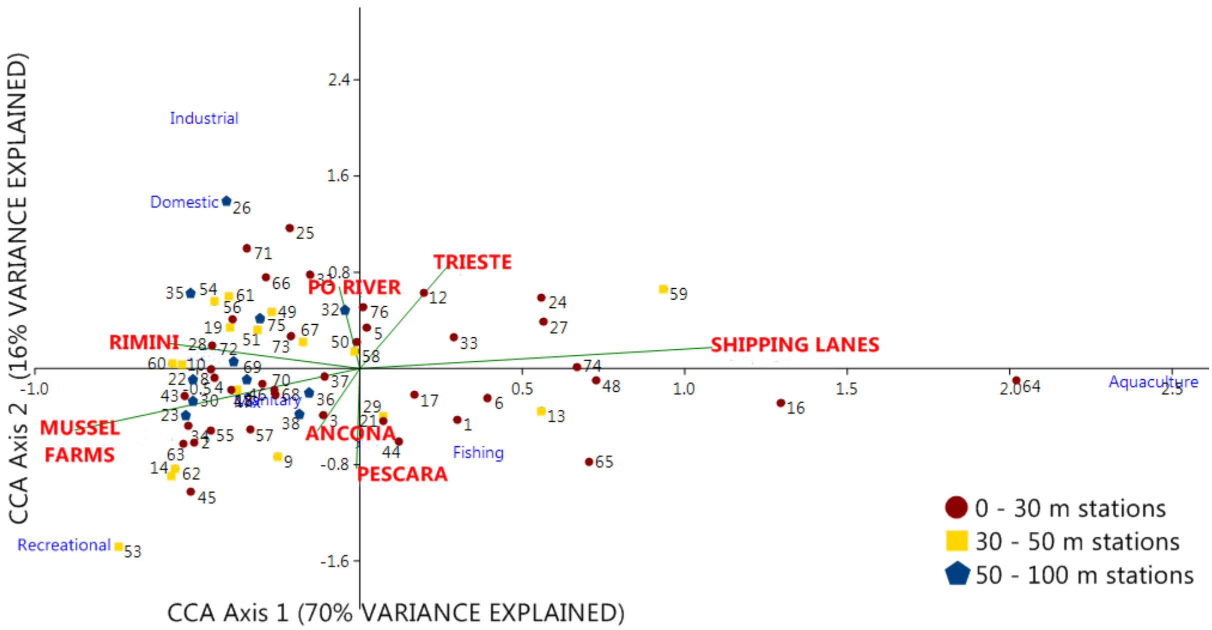


Figure 4.2.10. CCA ordination diagram (triplot) with litter densities grouped according to the activity of origin (blue labels) and litter sources (capital red labels and green lines). Dark-red points represent hauls in the depth stratum 0-30 m, yellow squares hauls in the depth stratum 30-50 m and blue pentagons hauls in the depth stratum 50-100 m.

Litter composition varied significantly among stations depending on the bathymetry. Results showed that stations from 30 to 50 m depth significantly differed from stations belonging to the other depth strata (Table 4.2.3). Similarity Percentages analysis (SIMPER) was used to identify which types of human activities primarily drove the differences among stations. This analysis identified that domestic, recreational and mixed activities were consistently responsible for a large percentage (> 70%) of the overall differences (Table 4.2.4).

Table 4.2.3. PERMANOVA analysis results and post-hoc pair-wise test between all pairs of groups. Significant comparisons ($p < 0.05$) are shown in bold red.

	Total SS	Within-group SS	Pseudo-F	P	Permutations N
Depth range	14.22	13	3.013	0.0009	9999

	0-30 m	30-50 m	50-100 m
0-30 m		**0.0013**	0.0528
30-50 m	**0.0013**		**0.0054**
50-100 m	0.0528	**0.0054**	

Table 4.2.4. SIMPER analysis of significantly different pairs of groups identified through PERMANOVA. The contribution of the three main activities causing the major dissimilarity are also reported.

Comparison	Overall avg. dissimilarity	Contribution %	Cumulative %	Mean abundance	Mean abundance
0-30 m vs 30-50 m	**67**				
	Mix	30.6	30.6	398	156
	Domestic	23.2	53.8	235	95
	Recreational	16.7	70.5	162	103
30-50 m vs 50-100 m	**64.3**				
	Domestic	33.4	33.4	95	338
	Recreational	23.4	56.8	103	219
	Mix	23	79.8	156	214

Since CCA showed that shipping lanes and mussel farms were the main drivers in litter composition, PERMANOVA analysis was performed testing these two factors. Results showed that stations closer to mussel farms significantly differed from stations closer to shipping lanes (Table 4.2.5). Similarity Percentages analysis (SIMPER) was used to identify which litter categories primarily drove the differences among stations. This analysis identified that synthetic mussel nets, plastic bags and sheets were consistently responsible for a large percentage (> 55%) of the overall differences (Table 4.2.6).

Table 4.2.5. PERMANOVA analysis results.

Source	Total SS	Within-group SS	Pseudo-F	P	Permutations N
Closest source	21.02	20.26	2.412	0.0051	9999

Table 4.2.6. SIMPER analysis of significantly different pairs of groups identified through PERMANOVA. The contribution of the ten main litter categories causing the major dissimilarity are also reported.

	Overall avg. dissimilarity	Contribution %	Cumulative %	Mean abundance	Mean abundance
Shipping lanes vs Mussel farms	**78**				
	Mussel nets	23.6	23.6	51.2	536
	Bags	17.4	41	170	302
	Sheets	14.5	55.5	159	214
	Other plastic	7.5	63	53.3	111
	Caps/lids	5.4	68.4	38.4	87.2
	Other mix	3.2	71.6	38.1	41.9
	Other rubber	3	74.6	13.7	45.8
	Processed wood	2.9	77.5	15.8	33
	Glass pieces	2.6	80.1	39.2	21.4
	Glass bottles	2.5	82.6	40	4.56

4.3 Macrolitter ingested by fish

A total of 260 fishes from 8 species representing 7 teleosts and 1 elasmobranch inhabiting coastal waters in the North-Western Adriatic Sea were dissected (Table 4.3.1). Analysis of their gastro-intestinal tract revealed that debris was present in the guts of 121 individuals, i.e. 47% of all fishes examined (Table 4.3.2). The percentage frequency of occurrence (%F) ranged between 25 and 64%, with *Mustelus* spp. showing the highest value. 51% of demersal species contained macro-litter. Analysing stomach and intestine separately, debris was found in the 26% of stomachs (%F ranged from 0 to 50%) and 31% of intestines (%F ranged from 25 to 43%), with *Mustelus* spp. showing the highest values.

Table 4.3.1. Fish species examined and main biological parameters recorded.

Species	No. of guts examined	Average fish length ± SE (cm) [range]	Average fish weight ± SE (g) [range]
Demersal	**108**		
M. barbatus	48	17 ± 2 [12.9-23.4]	63 ± 25 [25-180]
M. surmuletus	8	20 ± 2 [16.9-21.5]	117 ± 32 [73-171]
C. lucerna	26	24 ± 2 [20.4-26.7]	121 ± 34 [71-191]
S. solea	36	26 ± 3 [21.4-35.4]	187 ± 95 [105-502]
Benthopelagic	**119**		
P. erythrinus	30	17 ± 3 [7.8-21.7]	87 ± 39 [17-148]
S. aurata	35	20 ± 2 [16.6-28.6]	109 ± 44 [72-332]
Mustelus spp.	44	63 ± 18 [42.5-123]	1001 ± 1102 [206-6540]
Pelagic	**33**		
S. pilchardus	33	15 ± 1 [13-18]	25 ± 5 [15-42]
Total	**260**		

Table 4.3.2. Occurrence of ingested debris. Vacuity Index is the percentage of empty guts found among all guts examined. Percent frequency of occurrence (%F) is the proportion of the guts examined that contained debris.

Species	No. of non-empty guts examined	Vacuity Index (VI)	No. of guts with debris [%F]
Demersal	**107**	**1%**	**55 [51]**
M. barbatus	47	2%	25 [53]
M. surmuletus	8	0%	2 [25]
C. lucerna	26	0%	9 [35]
S. solea	36	0%	19 [53]
Benthopelagic	**118**	**1%**	**53 [45]**
P. erythrinus	29	3%	8 [28]
S. aurata	35	0%	17 [49]
Mustelus spp.	44	0%	28 [64]
Pelagic	**33**	**0%**	**13 [39]**
S. pilchardus	33	0%	13 [39]
Total	**258**	**99%**	**121 [47]**

In total, 199 items of debris were identified in the fish specimens examined. The number of debris items per fish ranged between 1 and 6 (2 ± 1 ingested pieces on average), with the highest value observed for *Mustelus* spp. (Table 4.3.3). Debris pieces size ranged from 1 to 25 mm (3 ± 3 mm on average) and colors varied, being black, blue and light blue the most common.

Table 4.3.3. Debris incidence and main characteristics.

Species	No. of guts with debris	Total No. of debris pieces found	Average No. of pieces per individual ± SE	Average debris length (mm) ± SE
M. barbatus	25	31	1.2 ± 0.5	4 ± 5
M. surmuletus	2	2	1 ± 0	2.6 ± 0.8
C. lucerna	9	11	1.2 ± 0.4	2.4 ± 0.8
S. solea	19	26	1.4 ± 0.7	3 ± 2
P. erythrinus	8	12	1.5 ± 0.8	3 ± 4
S. aurata	17	27	1.6 ± 0.9	3 ± 2
Mustelus spp.	28	73	3 ± 2	3 ± 2
S. pilchardus	13	17	1.3 ± 0.5	3 ± 3
Total	**121**	**199**	**2 ± 1**	**3 ± 3**

The most common category was plastic, representing almost the totality of debris items (> 99%), except for a piece of coal found in the stomach content of a *S. aurata* sample. The ingested plastic types were mostly represented by thread-like user plastic (95.5%), followed by hard fragments (2%), polystyrene pieces (1.5%) and sheets fragments (0.5%) (Fig. 4.3.1). Examples of some of these different types of debris are shown in Figure 4.3.2. *S. pilchardus* showed the highest heterogeneity among litter composition, while in demersal species the only litter category found was filament/fiber (Table 4.3.4).

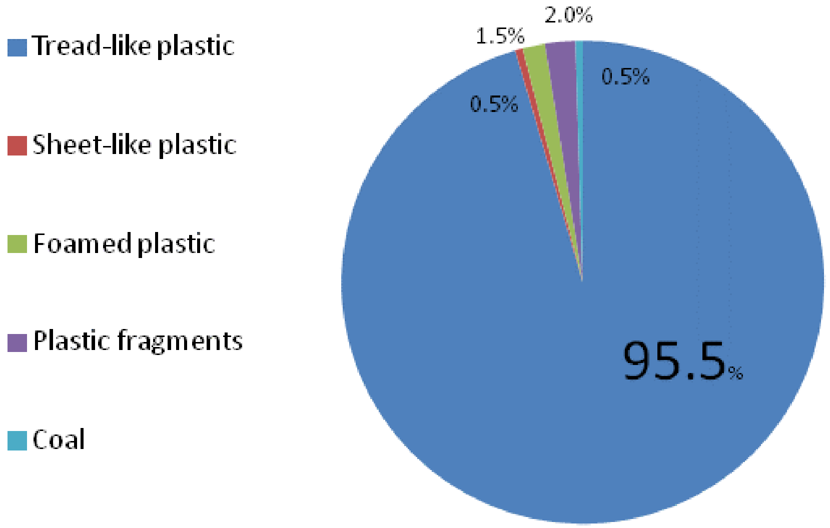


Figure 4.3.1. Composition of debris found in the guts. Percentages represent the total numerical abundance (%N) of litter items.

Table 4.3.4. Occurrence of different types of marine debris, categorized per species. Numbers in brackets indicate the relative percentage numerical abundance (%N) of litter categories found in the gut contents of examined fishes.

Species	Total No. of debris found	Tread-like plastic [%N]	Sheet-like plastic [%N]	Foamed plastic [%N]	Hard plastic fragments [%N]	Coal [%N]
M. barbatus	31	31 [100]	0	0	0	0
M. surmuletus	2	2 [100]	0	0	0	0
C. lucerna	11	11 [100]	0	0	0	0
S. solea	26	26 [100]	0	0	0	0
P. erythrinus	12	11 [91.7]	0	0	1 [8.3]	0
S. aurata	27	26 [96.3]	0	0	0	1 [3.7]
Mustelus spp.	73	69 [94.5]	0	2 [2.7]	2 [2.7]	0
S. pilchardus	17	14 [82.4]	1 [5.9]	1 [5.9]	1 [5.9]	0
Total	**199**	**190 [95.5]**	**1 [0.5]**	**3 [1.5]**	**4 [2.0]**	**1 [0.5]**

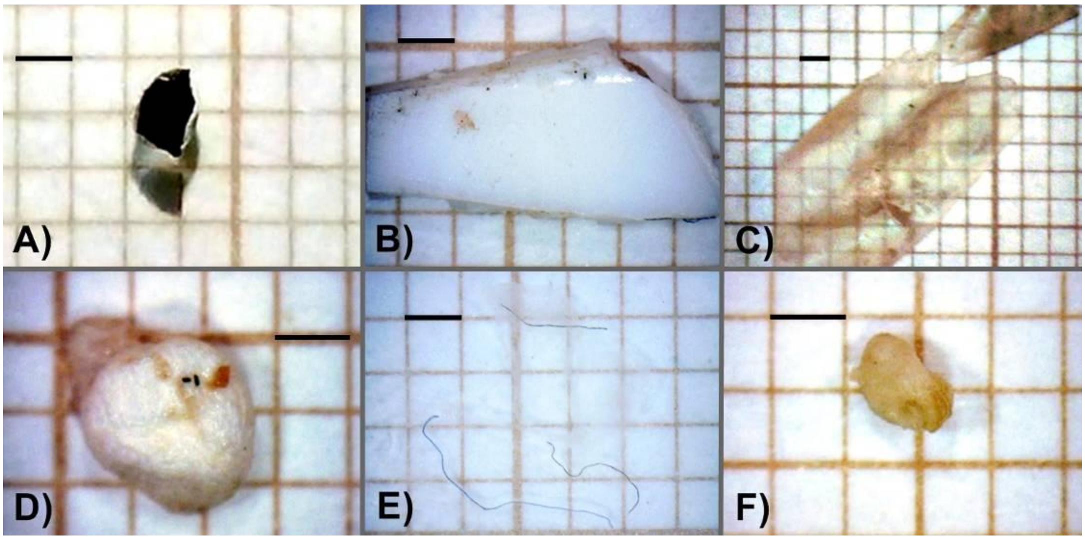


Figure 4.3.2. Examples of anthropogenic marine debris found in the guts of Adriatic fish species. **A)** Piece of coal (*S. aurata*); **B)** Plastic fragment (*Mustelus* spp,); **C)** Piece of plastic sheet (*S. pilchardus*); **D)** Polystyrene piece (*Mustelus* spp.); **E**) Fibers (*S. aurata*); **F)** Polystyrene piece (*S. pilchardus*). Scale bars are 1 mm.

5. DISCUSSION

5.1 Beach litter monitoring

5.1.1 Beach cleanliness

Densities of marine litter on beaches in the western Adriatic were similar to those reported from Slovenia coast (Laglbauer et al., 2014), and in Monterey Bay, USA (Rosevelt et al., 2013), placing in an intermediate position among beaches in other parts of the world (Table 5.1.1). Great differences in litter density were found between the present study and the study of Munari et al. (2015), although the surveyed area (Italian Adriatic coast) and the survey time were similar, and one of the beaches monitored was the same (Rosolina mare). This is due to the fact that in the latter plastic sheets, mussel nets and cotton bud sticks were not accounted as litter categories, despite their large abundance along the Adriatic coast.

In the present study, the visual descriptions of beach cleanliness provided by Alkalay et al. (2007) were consistent with the CCI values obtained. During the spring survey, the three sites ranked as “extremely dirty” or “dirty”, and in fact a lot of litter was visible on the beach. In summer, during the second survey, the visual appearance was quite better, except for Boccasette, which ranked again as “extremely dirty”. Among the three examined beaches, Boccasette in fact accumulated more marine litter than the others, even if no significant differences were found.

Though it was not possible to statistically compare seasons, differences in terms of litter abundance were evident, consisting in a reduced waste density from spring to summer (from 1.5 to 0.8 items/ m^2). This result is inconsistent with the general trend of litter accumulation found i.e. on Brazilian (Silva et al., 2015) and Balearic beaches (Martinez-Ribes et al., 2007), where debris contamination greatly increases during the tourist season. The outcome could be explained by the cleaning activities carried out at the beginning of the tourist season, precisely at Torre del Cerrano, where a sort of hand waste collection took place in June. Yet, a low accumulation rate after litter removal during the spring survey cannot be excluded.

Table 5.1.1. Comparison of mean litter densities and plastic abundance from beaches throughout the world.

Country	No. surveyed beaches	Avg density (items m^{-2})	Plastic (%)	References
ITALY (W Adriatic)	3	1.15	92	This study
ITALY (N W Adriatic)	5	0.2	81	Munari et al. (2015)
SLOVENIA	6	1.51	64	Langlbauer et al. (2014)
RUSSIA	8	0.2	55	Kusui and Noda (2003)
JORDAN	3	4	50	Abu-Hilal and Al-Najjar (2004)
JAPAN	18	3.4	73	Kusui and Noda (2003)
TAIWAN	6	0.15	86	Kuo and Huang (2014)
PAPUA NEW GUINEA	20	15.3	90	Smith (2002)
AUSTRALIA	6	0.1	90	Cunningham and Wilson (2003)
PANAMA	19	3.6	82	Garrity and Levings (1993)
BRASIL	5	4.98	90	Widmer and Hennemann (2010)
CHILE	43	1.8	n.d.	Bravo et al. (2009)
USA (Monterey)	12	1	68	Rosevelt et al. (2013)

5.1.2 Beach litter composition

The vast majority (over 90%) of marine litter collected on all the three beaches was plastic, the most common type of debris found on beaches throughout the world, including inaccessible islands (Ocean Conservancy, 2010), in accordance with results from the literature (Table 5.1.1). The main reason for its great abundance is that plastic is used in almost all human activities (professional and recreational), together with its long persistence and ease dissemination in the marine environment (Derraik, 2002). Among plastic, sheets derived from industrial packaging, unrecognizable hard fragments and polystyrene pieces were the most frequently collected type of litter, a finding consistent with the results found in Australia (Cunningham and Wilson, 2003), Russia and Japan (Kusui and Noda, 2003), Carribean Sea (Garrity and Levings, 1993) and many other parts of the world. The second plastic category with the highest occurrence was mussel nets, polypropylene tubular nets for mussel grafting and breeding. Mussel nets are often accidentally lost at sea due to storms which cause their detachment from installations or due to farmers negligence or intentionally discharged (Mare s.c.a.r.l., 2014), and their presence is a good indicator of pollution from local sources. At Boccasette, the site with the highest concentration of mussel nets (0.3 items/m^2 in spring; 0.2 items/m^2 in summer), the nearest offshore mussel farm was in fact at a distance of about 4.5 Km from the beach, while at Rosolina mare (0.1 items/m^2 in spring; 0.04 items/m^2 in summer) at 7 Km and at Torre del Cerrano (0.08 items/m^2 in spring; 0.005 items/m^2 in summer) at 12 Km. The third most abundant plastic category was cotton bud sticks, most likely coming from inland. Cotton bud sticks are often mistakenly throw into the flush and as they are small enough to pass through screens at sewage treatment works, they are now a common item littering coastal shore waters and beaches (Martinez-Ribes et al., 2007; Marine Conservation Society, 2008), even in the Adriatic. Their presence seems not to decrease, despite the national legislative provision (Article 19 of D.Lgs.93/01, issued on March 2001) prohibiting the marketing in Italy of non-biodegradable cotton bud sticks, raising many concerns regarding their persistence in the environment.

Due to the plastic limitation policy recently implemented by the Italian Government (National Act D.Lgs.91/2014, issued on June 2014, prohibiting the marketing of non-biodegradable shoppers), the percentage of plastic bags was fairly limited (1.2-1.5%) compared to other regions i.e. Jordan (27-29%, Abu-Hilal and Al-Najjar 2004) and Panama (15%, Garrity and Levings,1993).

5.1.3 Beach litter origin

Determining the type and source of marine litter on beaches is certainly important to develop actions aimed at minimizing its presence in the environment. It is widely reported that most marine litter comes from land-based rather than sea-based sources (Rees and Pond, 1995; UNEP, 2005). According to the analysis of data collected between 2002 and 2006 by PNUE/PAM/ MEDPOL (2009), 52% of marine litter in the Mediterranean Sea originates from shoreline and recreational activities, 40% from smoke-related activities, 5% from boat activities, 2% from dumping activities, and 1% from medical and personal hygiene. Present results showed that, in Western Adriatic beaches too, the majority of marine litter comes from land-based sources (77%), but relative percentages were different. Excluding categories which have indefinite or multiple sources, the origin of marine litter was primarily shoreline and recreational activities, accounting on average for 32% of litter sources, a value much lower than the global average (68%) reported in 2010 by Ocean Conservancy, or the Mediterranean average. This source was followed by medical/personal hygiene items (20%), dumping activities (15%), and smoking-related activities (10%). The remaining 23% consists of fishing-related debris.

The high percentages of *in situ* deposited litter found in all surveyed beaches were likely caused by the high number of visitors, more than 700,000 annually on average (2013 data; http://statistica.regione.veneto.it). In free access beaches, like those considered in this study, tourists carry food and recreational equipment that may not be properly disposed of, leading to such a high percentage of recreational and smoke-related litter. In all sites, most common items derived from recreational activities were plastic and glass bottles, their caps and lids, food containers and wrappers, picnic supplies (plastic dishes, cutlery, cups and trays), shotgun cartridges and smoking-related items (cigarette butts, lighters, cigarette boxes and plastic packaging), the latter with a density much lower than indicated for the Mediterranean and the neighboring Slovenian coastline (Langlbauer et al., 2014). Even if total litter collected decreased from spring to summer survey, it is important to underline the increase of beach-goers littering, consisting in a great abundance of paper bags, tissue, smoking-related items, especially at Torre del Cerrano, which is a popular summer destination, where cigarette butts density increased 10 times from spring to summer season.

Dumping activities also contributed substantially to litter composition stranded on study beaches. At Boccasette and Rosolina mare, debris from dumping activities consisted mainly in household trash (chemical and detergent bottles, caps and containers) likely transported from inland by the large rivers (Po and Adige river, respectively) flowing into this coastal area, indicating an inefficient municipal handling of waste. At Torre del Cerrano, dumped debris mainly consisted in foam sponge, strapping bands, cables and other construction material, likely coming from building activities in the surroundings and direct discharge by workers.

Contrary to Munari et al. (2015) and to the Mediterranean average, medical/personal hygiene items represented a relevant percentage in litter composition (20%). The most representative category among sanitary debris was cotton bud sticks, covering a percentage of 80% in both surveys. Boccasette showed the highest density of medical and personal hygiene items, likely linked to its vicinity to Po river mouth.

Lastly, ocean/waterway activities contributed for 23% to total litter stranded on Western Adriatic beaches, a much greater percentage compare to the Mediterranean (5%; PNUE/PAM/MEDPOL, 2009) and worldwide averages (8%; Ocean Conservancy, 2010). It must be stressed that almost the totality of maritime waste collected consisted of mussel nets either in spring (95%) or summer (93%), whilst strictly fishing-related debris (buoys, nets, fishing lines) represented a very small percentage, disproving the common perception that fishermen are highly responsible for marine littering (MARLISCO, 2013).

5.1 Benthic litter densities, characteristics and origin

Using *rapido* trawl nets it was possible to survey the abundance, distribution, density and typology of marine litter items found on the seafloor in the Northern and Central Adriatic Sea. Litter densities in terms of weight confirmed data of the 2011/2012 SoleMon survey (Strafella et al., 2015), whereas densities in terms of numbers was almost three times the total litter density found by Galgani et al. (2000) during BIOMAR survey in 1998 (Table 5.2.1). Comparing seafloor litter densities recorded in this study with other studies worldwide, it can be assumed that the Adriatic shows the greatest seafloor litter pollution not only among Mediterranean regions, but among worldwide seas too (Table 5.2.1). This extremely high density could be explained by the combination of high anthropogenic pressures (e.g. densely populated coastline, intensive shipping, massive tourism, fishing and aquaculture) and environmental features, since the Adriatic is a semi-closed basin with a limited water exchange, negligible tidal flow and massive river flow inputs. It is estimated that the total annual input of plastic in the Adriatic Sea was 10,000- 250,000 tons in 2010 (Jambeck et al., 2015), mainly originating from the Western coastline, with “hot spots” found in the Po Delta, Venice, Chioggia and the Reno Mouth (Liubartseva et al., 2015).

Notwithstanding, it is relevant to highlight that the sampling gear used in the present study may have a different (probably higher) performance respect to bottom otter trawl nets in collecting marine waste, being appositely planned to capture benthic organisms (Strafella et al., 2015).

Table 5.2.1. Densities and proportion of plastic in benthic marine litter in the Adriatic, Mediterranean and worldwide seas, collected from bottom trawl surveys. N: Northern; C: Central; S: Southern; W: Western; E: Eastern.

Sea	Litter density	Plastic %	References
N & C Adriatic	913 items/Km^2; 82 Kg/Km^2	80; 62	This study
N & C Adriatic	85 Kg/Km^2	34	Strafella et al. (2015)
Adriatic	378 items/Km^2	70	Galgani et al. (2000)
Gulf of Lions	143 items/Km^2	71	Galgani et al. (2000)
W & S Greece	165 items/Km^2	56	Koutsodendris et al. (2008)
Baltic	126 items/Km^2	36	Galgani et al. (2000)
W Pacific	185 items/Km^2	54	Kuriyama et al. (2003)
E Pacific	30 items/Km^2	23	Keller et al. (2010)

Similarly to what has been reported for other European seas (Galgani et al., 2000; Koutsodendris et al., 2008), higher quantities of litter were found in the coastal areas rather than the open sea, especially in front of the largest river mouths, coastal cities and aquaculture installations. This suggests that the large volume of litter coming from inland or coastal sources that enters the marine system mainly concentrates in shallow waters, and only a small percentage reaches deeper waters. In deep waters, high litter densities hotspots are likely associated with the most congested shipping lanes, indicating an additional litter input to the basin.

The identification of the actual input of litter in the Adriatic Sea is critical. It is estimated that 40% of the marine litter enters the basin through rivers, 40% through coastal urban populations and the remaining 20% is derived from shipping lanes (Lebreton et al., 2012). Mussel farms are also an important input of debris in the basin, which cannot be ignored and excluded from analyses.

Plastic constituted the main portion of collected litter, consistently to Mediterranean and worldwide findings (Galgani et al., 2000; Derraik, 2002), due to its extensive use, buoyancy and poor degradability. Indeed, this category appeared homogeneously distributed throughout the Adriatic Sea and mainly consisted in bags and sheets, which are able to travel long distances before sinking, making extremely difficult to identify their actual source (Pham et al., 2014). Mussel nets represented an high percentage (20%) of plastic found, and derived from the intensive mussel farming along Italian coast, with over 263 mussel farms (Prioli, 2008) and an annual production of about 63,500 tonnes, corresponding nearly to 50% of the national shellfish culture production (FAO FISHSTAT, 2005). Conversely, synthetic fishing-related items including nets, ropes and monofilament lines, clearly indicate a fishery-based source and occurred only for the 2% of total collected plastic, contrasting general findings in the Mediterranean that found much higher percentages (Ramirez-Llodra et al., 2013; Güven et al., 2013). The remaining portion of plastic litter (bottles, caps, sanitary items, etc.) could be originated from land-based sources, including domestic, recreational and industrial activities. However, it cannot be excluded a release from vessels, as evidenced in this study by the overlapping of plastic distribution and some of the main shipping routes in the Northern and Central Adriatic (Strafella et al., 2015).

Metal and glass (such as cans, drums, bottles, jars, etc.) usually sink rapidly, and thus do not travel long distances, so they are most likely found close to their sources. They occurred mainly in coastal areas, close to the harbors' entrances, as well as in the open sea along the shipping routes in the middle of the Adriatic basin (international waters), suggesting that they were probably originated from marine-based sources including ferries, merchant vessels and recreational boats.

Rubber made a little percentage of the total collected litter, and included categories such as boots, bobbins and car tyres, all likely indicating a fishery-based source. Indeed, fishermen use to employ the latter as fishing boat fenders (Koutsodendris et al., 2008).

Worked wood was mainly collected from coastal areas, and its distribution may be related more to surface currents and general water circulation rather than its source, however its density was generally low.

Finally, objects included in the category ''mix'' (cloth and other materials) are not good indicators of litter sources since they might have been originated both from land and marine-based sources. Mariners use cotton wastes to clean the engines of the boats, while pieces of cloths could also have originated from land and vessel-based activities.

From CCA it emerged that off-shore stations (30-100 m) have a similar litter composition, being abundant in litter originating from domestic and recreational activities and poor in aquaculture-related items, mainly driven by the presence of important shipping lanes. The 50% and the 64% of litter categories belonging to domestic and recreational activities, respectively, is attributable to vessel-based sources, making clear the role of marine traffic in litter distribution and composition. Conversely, coastal stations (0-30 m) were more heterogeneous in terms of litter composition. This may be due to the overlapping of several litter sources in coastal areas.

However, shipping lanes are not the only source driving main differences in litter quali-quantitative spatial distribution in the study area. CCA results showed indeed that another important factor was mussel farms. PERMANOVA analysis confirmed the finding, showing that stations close to mussel farms significantly differed from stations close to the most congested shipping lanes, and the litter categories driving the major differences consisted in mussel nets, followed by plastic bags and sheets from packaging. The last two may originate from almost all human activities and may be introduced into the basin from any possible source, and thus are not a good indicator of litter inputs. On the contrary, mussel nets clearly originated from a sea-based source that is aquaculture. Thus, pollution derived from mussel nets is a serious problem not only along Italian beaches, but also on the seabed of the North and Central Adriatic sea, especially in coastal waters, raising the need of focused management measures. Finally, it is worth noting that glass bottles, even if contributed only for the 2.5% of the total dissimilarity among stations close to shipping lanes and those close to mussel farms, were almost ten times more abundant in the former. This result makes clear the vessel-based source of this category, as a result of the indiscriminate dumping by tourists and sea users.

5.3 Macrolitter ingested by fish

The data gathered in the present study give, for the first time, indications on the impact of debris on several commercial fish species encountered in coastal waters of the North Western Adriatic Sea. Findings showed that the presence of debris in fish guts was not as rare as for deep-waters fishes off the Eastern Ionian Sea, where anthropogenic items occurred in only 1.9% of examined organisms (Anastasopoulou et al., 2013). In the present study, 47% of fishes were found to ingest litter, which is consistent with values reported for planktivorous fishes in the North Pacific Central Gyre (Boerger et al., 2010), the ocean convergence zone that accumulates plastic debris from the entire North Pacific reaching concentrations of over 300,000 pieces per km^2 (Moore et al., 2001).

It must be stressed that more than 95% of litter items found in the gastro intestinal contents consisted in fibers. This result might be biased by air-borne litter contamination during laboratory processing (Woodall et al., 2015). Despite the procedures to avoid external pollution and the use of the blank sample, potential contamination of samples by airborne microfibers subsisted, especially during fish dissection or gut contents extraction, reducing the robustness of results. If fibers were excluded from analyses, debris ingestion would occur in 3.5% of total fishes dissected, a value consistent with the Eastern Ionian Sea incidence, yet a bit higher. The difference may be related to species environment, being coastal waters more sensitive to litter pollution than deep waters (Pham et al., 2014).

In the present study, almost the totality of ingested litter consisted in plastic, confirming worldwide findings (Boerger et al., 2013; Miranda and de Carvalho-Souza, 2015). Results are explained by the fact that plastic usually fragments into smaller and easily ingested pieces compared to other categories, such as metal and glass (Anastasopoulou et al., 2013). Plastic in fish gut contents mainly derives from direct ingestion because of misidentification of debris items as natural prey, or accidentally during feeding from sediment and water (Koutsodendris et al., 2008). Extent and type of ingested debris categories may be related to feeding behavior. In the present study, benthic detritivorous species ingested only thread-like plastics, whereas benthopelagic and pelagic species showed higher heterogeneity among litter composition, especially *S. pilchardus*, which swallowed almost all debris categories. This small pelagic species plays a key role in the Adriatic Sea, from both ecological and socio-economic points of view, being a common prey for larger pelagic predators and one of the most important commercial species of the basin, with an estimate annual catch of about 18,000 tonnes (Santojanni et al., 2005). Although it is not possible to fully understand the effects of ingestion yet, potential concerns may arise. If fish are not able to excrete the ingested plastic, the accumulation of non-nutritive elements may cause malnutrition and eventual starvation, which could lead to significant reductions in fish populations.

One of the major hazards associated with plastic litter in the marine environment and, consequentially, on marine organisms, is the enhancing of the accumulation and bioavailability of Persistent, Bioaccumulative and Toxic (PBT) substances (such as PCBs and DDT), in addition to toxic chemicals that have been added, during the production procedure, to increase the performance of the plastic (such as phthalates, nonylphenol, bisphenol A, brominated flame retardants) (Romeo et al., 2015). Laboratory experiments showed that plastic particles and toxic compound absorbed transferred from gut to tissues of mussels (Browne et al., 2008) and fish (Rochman et al., 2013), and many other organisms at various trophic levels (Teuten et al., 2009) causing severe impacts, including liver toxicity and pathology, endocrine disruption and behavioral, physiological, and metabolic alterations (Meeker et al., 2009). Finally, micro plastic particles can be transferred from one trophic level to the next (Farrel and Nelson, 2013; Romeo et al., 2015), posing an additional threat to fish and their predators at higher levels of the food chain, such as tunas, marine mammals, seabirds and humans ultimately (Engler, 2012).

6. CONCLUSIONS

The present thesis represents the first step for a large-scale, standardized assessment of litter pollution in the Adriatic region, investigating both environmental and biological compartments. Results from this study strongly indicate that most marine litter in the three beaches and on the seabed comes from very local sources, that is mussel farms and recreational users of the coast and the sea. Besides mussel nets coming from mussel farming installations, high proportion of litter on the three beaches has been directly deposited there by beach users, and high proportion of litter on the seabed has been directly dumped from ships, boat and ferries.

Findings may provide a baseline to set the necessary measures to manage marine litter deposition and accumulation in the Western Adriatic region and to minimize such type of anthropogenic pollution. Continued and intensified public education campaigns and activities aiming at reducing litter at sources, as well regulations for mariners, fishermen and shellfish farmers, are required. However, continuous monitoring of marine litter in all environmental compartments is important in order to assess the effectiveness of the international regulations. Abundance, composition and degrees of influence of the litter sources are very important information for municipal and port authorities to formulate plans and actions related to prevention measures, and ultimately achieve the GES in the EU marine waters by 2020 provided by the MSFD.

As for impacts of plastic debris on marine biota, despite the possible bias coming from air-borne contamination, the evidence of plastic ingestion by commercial fish subsists. Further work is needed on a higher number of organisms, species and trophic guilds to better assess the distribution of plastic along food webs, to investigate the potential for pollutant transfer to higher trophic levels, with potentials concerns also for human consumers.

Finally, as litter knows no political borders, a trans-boundary vision of marine litter issue is necessary in order to set effective decision making, towards litter free biota, coasts and sea.

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